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SOME PROBLEMS OF HUMAN RACIAL DEVELOPMENT AND MIGRATION[1]

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I HAVE been asked to discuss "Some Problems of Human Racial Development and Migration" in the space of a half hour. I once had a professor who urged "take time to be brief," but there are so many angles to this subject and it covers so much time and space that the best I can hope to do is to indicate a few of the problems.

My difficulties begin when I use the term *human*. Where is the dividing line between prehuman and human? Just about the time of the Dayton trial came the discovery of Australopithecus. A London journal came out with an illustrated article calling this the oldest find of fossil man in Africa or perhaps in the world. American papers copied and one of our British colleagues was so fearful I might use the material in my testimony that he sent me a long message saying the find was not man but an ape far advanced toward man.

How human does a "man-ape" have to be before we class him as human? Does the distinction lie in cranial capacity? If so, the six-year-old Australopithecus had already passed out of and beyond the range of Anthropoid apes of like age.

Is it in proportions of the brain? If so, this youngster showed very human traits in the high degree of development of parietal bosses and in expansion in the temporal portions. Or is it in teeth? Here the baby teeth show intermediate characters.

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[1] **Address of the retiring president, American Society of Naturalists, Cleveland, Ohio, September 14, 1944.**

Probably we would agree that we must use a complex of traits in making the distinction between man and the apes.

At the time Australopithecus was found it was considered as probably Pliocene in time! Now in the same area of Africa we have closely related adult forms which come so far into Pleistocene that it is clear we are dealing with a line of apes that never quite arrived. They present a far development or experiment toward man, but for some reason died out without becoming *homo*.

But what is *homo?* When the much disputed teeth, skull cap and femur of *Pithecanthropus erectus* were first examined by a group of specialists, part said that if all belonged to one individual he was a primitive type of man; others said "giant baboon" and still others said "an intermediate form—an erect ape-man." The additional discoveries of Dubois and now the materials offered by von Koenigswald and Oppenooth—jaws, more teeth, additional femora, three adult and one baby skulls—leave us in no doubt that we are dealing with man. We have a complex of traits falling closer to man than to apes.

The closely related Sinanthropus forms found in China are likewise accepted as fossil men and here an additional claim is added—the use of fire and possession of tools. Can we use evidences of man's intelligence and creative ability in our attempts to classify?

Another set of questions is raised when we inquire of the time and place of man's arrival. By referring again to teeth and fragmentary jaws we can say that beings possibly ancestral to man and the Anthropoid apes had appeared in Miocene times in India and in Europe. We can go further and say that the flora and fauna with which the Sivapithecus beings of India were associated extended toward Europe and across India and Burma to the southeast as far as Java—thus extending the probable range of these "ancestral" forms. It is important to note that in later horizons we find Pithecanthropus at one end of this range while at the other end, in Europe, claims for early man are set up by the discovery of Heidelberg, Piltdown and Swanscomb. Man at both ends of this arc but no equally ancient finds in between.

Where are the evidences of the developing forms which must have spanned the interval between Sivapithecus, Dryopithecus and the human forms just mentioned?

To-day it s generally conceded that Pithecanthropus is not earlier than second interglacial—perhaps later—while no fossils from Europe or Africa can lay clear claim to an earlier birthday.

A vast time interval extends between these possible ancestral forms of Miocene times and the first forms on which we can agree as being man.

For more than three decades Pithecanthropus stood alone in Asia in the battle with the fundamentalists. During that period some came to doubt the reliability of his teeth; others insisted that the very straight femur never supported the very primitive skull cap, and—unkindest cut of all—his discoverer came to class him as a giant gibbon. Then came the new discoveries. Cast doubt on any single feature or combination of features of Pithecanthropus, and the recently excavated materials rise to confound you.

Still more convincing are the additional discoveries in this part of the world. Toward the end of Tertiary times the easy line of movement between India and Java seems to have been broken and we no longer speak of the Siva-Malayan fauna. In its place comes the Sino-Malayan, which extends from Java to Pekin. Now with the Sinanthropus finds we have early fossil man of nearly the same time period at both ends of *this* arc.

Sinanthropus and Pithecanthropus seem much alike, yet because of the circumstances surrounding the discovery and description of the first Sinanthropus tooth we are confronted with a new genus and species. Should we continue to recognize similar forms under diverse names because of historical accidents, or should we accept recent suggestions and call the Chinese finds *Pithecanthropus pekinensis?*

Claims for second interglacial man in Europe are fairly well substantiated by the finding of man-made objects in the strata of that time. It also seems possible that the famous Heidelberg jaw belongs to this period. Of this jaw we need only say that except for size it seems directly in line with the jaws of the later Neanderthalers.

Another find sometimes assigned to this period is Piltdown. The whole subject is so filled with question marks that most students of human history are in doubt whether we should bless the painstaking efforts of Charles Dawson, who recovered the fossils, or should berate him for dragging this skeleton into the family closet.

The deposit in which the materials were found is an alluvial fan claimed by some to be second interglacial, by others as third glacial. Some of the materials washed in are doubtless from Tertiary deposits but others belong to the last stages of the formation.

Workmen taking out materials for road repairs turned up pieces of a human skull. These came to the attention of Mr. Charles Dawson, who, in company with Sir Smith Woodward, carried out the search which resulted in the much disputed fossil. The workmen's pit extended four feet into the gravel and it was from this level that the crushed fragments of a woman's skull, half of a lower jaw, two molar teeth and a canine tooth and nasal bones were taken. With or below the skull was a bone tool cut to a rude point from the leg bone of an ancient type of elephant (Meridionalis), also some crude stone chips.

The skull and jaw do not bear evidence of having been water carried, as is the case of the tertiary bones in the deposit. They are badly crushed, but it is not certain whether this was due to ancient causes or to the workmen. They lie so shallow they could be intrusive from above—a suspicion which is strengthened by the association with the bone utensil and eoliths. On the other hand they appear, in color and stage of fossilization, to belong to the deposit.

Finally we come to the fossil itself. The bones of the cranium are unusually thick and there are some other hints of primitiveness, but taken as a whole the skull is very modern. It even lacks a suggestion of the huge supraorbital ridges which characterize Neanderthal and the early Asiatic finds. Yet the jaw appears to belong to a fossil chimpanzee, and this is further confirmed by the teeth.

The combination of an advanced skull with an ape's jaw proved too much for many scientists, yet a second find of portions of a skull and a lower molar of the same type as with the jaw does give aid and comfort to the reconstruction called *Eoanthropus dawsonii.*

The skull and jaw may belong to the same individual—a difficult situation even for asymmetrical evolution to explain. The association may have been fortuitous—a hard thing to explain in view of the second find. We may be dealing with a burial of modern man, who by chance was placed in a deposit containing the jaw of a fossil chimp. But no other fossil chimpanzees have been reported from England.

All this is bad enough, but now appears a group of scholars which argues that Piltdown is an early representative of *Homo sapiens* who had reached England as early as or earlier than the Neanderthalers.

To strengthen their claim they point to the very modern skeleton of Galley Hill man, to the fragmentary skulls found at London and St. Edmunds, to the east African discoveries of Leaky and finally to the Swanscomb fragments. Each and all of these are in question either as to the age of the deposits in which they lie, or as to their assignment to *Homo sapiens.*

Frankly we don't know what to do with Piltdown man—who by the way is a lady—we can't dismiss her—him—and we can't accept her credentials. We agree with Howells when he says that Piltdown has scotched more attempts to draw up a family tree for man than all the other fossils put together.

The story of Neanderthal man during the fourth and last major glaciation is well known. We know a good deal about his life and his physical type. It had long been suspected that he occupied Europe during the third interglacial, and the very Neanderthal-like jaw of Heidelberg suggested that his ancestors had appeared there by the second interglacial.

Recent discoveries show Neanderthaloids at Ehringsdorf, at Steinheim and near Rome in third interglacial. Part of the Mt. Carmel people likewise indicate a Neanderthal element in Palestine at this time, while the very Neanderthal-like people known as *Homo soloensis* recently discovered in Java, along with other finds, make it clear that man conforming rather closely to this type was widespread over Europe, Asia and possibly Africa by the end of the third interglacial.

But was he the only human of that period, and if so what of the ancestry of *Homo sapiens?* We have already noted the very questionable family tree built up through Galley Hill, Swanscomb, Piltdown and Kanjera series.

You all recall the dispute between Drs. Hrdlička and Elliott Smith following the Huxley lecture of 1927. Hrdlička indicated that *Homo sapiens* had developed directly out of Neanderthal, perhaps in Europe. Smith was firm in his rebuttal.

Just how closely are we related to Neanderthal?

The Steinheim skull, of early third interglacial, is accepted as Neanderthaloid, yet in skull height and more retracted face shows development toward later man. If Swanscomb isn't *Homo sapiens* then he shows development in that direction. The discoveries of Garrod and McCown in Palestine seem to indicate that *Homo sapiens* had already appeared and had intermixed with Neanderthal, or we are there dealing with a group recently branched off from that parental stem.

Another hint of what was going on about this time is supplied by the recent finds in Java. *Homo soloensis* is probably late third interglacial; he certainly is very Neanderthal-like. He may be descended from Pithecanthropus; he probably is ancestral to the later Wadjak fossils. These seem to tie up with the fossil finds in Australia and thus to the modern Australoids. But the Australians are *Homo sapiens.* The evidence is not absolute, but the hints are clear that Neanderthal may be much more closely related to us than we have been accustomed to think.

The appearance of Cro-Magnon in Europe during the waning of the last glaciation is ample evidence that man of our species had undergone a long period of development and specialization outside Europe and that he entered as a well-defined physical type quite different from Neanderthal.

That the classical Cro-Magnon with his large stature, long-narrow-high head and short, wide face was not the only representative of the species is clearly demonstrated by the discovery of the Grimaldi skeletons, the appearance of Brünn man and the variant types of skulls found at Oftnet and Mugum.

Apparently the question of sub-species and race is raised as soon as *Homo sapiens* is definitely known.

When I chose this topic I had expected to devote half my time to the modern races. Now my time is nearly finished and I have only alluded to modern man.

We can excuse our uncertainty regarding the fossil record by the scanty nature of the evidence, but problems do not cease as the evidence accumulates.

We accept the idea that all living groups of mankind belong to one and the same species and that the separation into sub-species and races probably goes back toward the end of the Pleistocene. The ways of classifying still lead to some confusion, but I believe the majority will accept the scheme whereby the species is first divided into three main stocks or sub-species—Negroid, Mongoloid and Caucasoid—and these are further subdivided into races, subraces and so on down.

For such a classification we must use a large number of traits to distinguish each division; putting together those who are alike and separating them from those who differ. We assume that certain traits—such as the curly or frizzly hair, broad flat nose, projecting face and everted lips of the Negroids—are old and entrenched and thus serve to distinguish them from Caucasoids and Mongoloids. But inside these divisions other groupings appear which serve to set up the races—such as Nordic, Alpine and Mediterranean. They possess most of the distinguishing traits of the Caucasoids and in addition have still others probably of more recent origin.

The probable causes of change are familiar to all—mutations, isolation, inbreeding, natural and social selection, possible effects of environment and so on. Here it need only to be recalled that archeology shows us that until the development of agriculture and animal husbandry, mankind was in small hunting and seed-gathering groups, much on the move. Under such conditions variant forms had a much greater chance of becoming established, while natural selection worked more drastically to eliminate those unfit for the conditions under which a group was then living.

Granted all this there are still many unsolved problems. For instance, most people think of Africa as the homeland of the Negro. But large and small Negroes are found not only in Africa but in New Guinea on the other side of the world. Traces of this people occur in the present populations or in archeological horizons along the coasts all the way between. It is extremely unlikely that environmental conditions at the two peripheries could independently produce the complex of traits by which we define the Negroids. There is much to suggest that, with the possible exception of the Bushman, all the Negro groups are rather recent intruders into Africa. If this is true we should perhaps look for origins somewhere toward north India.

We may imagine a time when the various isolated Caucasoid races were relatively pure and we can trace their movements with some assurance from the general region of the Aral-Caspian seas toward Europe, Asia and

India. Mutations reinforced by natural selection may have established a blond grouping in the Baltic area, while reverse conditions may account for the dark skin color of the Aryan speaking peoples of North India, who are otherwise close to the Mediterraneans.

The dividing line between the round-headed Alpine Caucasoids and the western Mongoloids raises questions of relationship other than race mixture.

With race mixture our problems really begin. Mixture is old and in many regions is very thorough. It has come about through migrations, invasions, war, slavery and countless other causes.

One group urges against mixture—especially of very diverse races. Claims of resultant sterility, of production of monsters, of lowered quality of body and intellect are met with equally valid evidences of hybrid vigor, of increased fertility and of superior physical types—due to mixture.

Biologically, race is an established fact, and this leads to claims for superior and inferior, for innate characters of race and so on. Such claims are dubious, but human attitudes do affect the mental and cultural development of whole groups.

Those of you who deal with other animals don't realize how fortunate you are. You can put your subjects into a cage; you can control their mating and you can watch results over several generations. No such happy situation faces the student of man. He is a slow breeder; he refuses to conform in his mating habits; and you can't lock him up for long. Paleontologists may say "if these bones could only speak," but if we judge by living man, most information on racial matters that the talking bones would give would be misinformation.

I have exceeded my time. I have only begun to touch on problems, but fortunately these are problems raised by increasing knowledge concerning man. A century ago speculation was rampant—to-day at least we have enough information so we can ask questions.

BIOLOGY AND HUMAN PROGRESS[1]

BIOLOGY AND THE PUBLIC MIND

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THE general topic proposed for our discussion to-day, "Biology and Human Progress," is a significant departure for these annual symposia of this time-honored Society of Naturalists. That departure is evidently in keeping with the tone and spirit of other sessions of the association this year and is, of course, amply justified.

Momentous world events, that are uppermost in the minds of men everywhere, continually remind us that this is a grave, crucial period in man's long, eventful, perilous history. A complacent view of our species history might be that having thus far managed somehow not only to survive but also slowly to gain dominion over the earth, the sea and the sky, we may be assured that nothing can ever finally prevent man's further progress.

The stark fact is, however, that twice within the memory of those here to-day, the nations of the earth have again repeated mankind's warring history, have again resorted to armed force as the means of deciding human issues, of solving human problems. And we are now well aware that for cost in lives and treasure, for sheer brutality and utter devastation, the five years now of this second World War have far surpassed that of World War I and so of any war of recorded history.

Lloyd Stratton, veteran of the last war and now chief of the British Associated Press, recently wrote from the Normandy bridge-head: "I saw nothing massed on the Western front in 1918 that in magnitude and power equalled that now concentrated on this small Normandy front. In the Caen area and around St. Lo, are towns as totally destroyed in one brief all-out offensive as occurred in the previous war after three years of back-and-forth fighting.''

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[1] Papers from a symposium presented at the meeting of The American Society of Naturalists during the sessions of The American Association for the Advancement of Science at Cleveland, Ohio, before a joint session with the American Society of Zoologists and the Botanical Society of America, September 14, 1944.

We over here can read such items and hear such reports, yet we can not grasp their full import. Fortunately for us, we are too far away from it all, in a fortunate land now made secure from the death and devastation of other war-stricken peoples and lands. Fortunately, perhaps, we can learn only through others of these depths of man's inhumanity to man—of entire cities that are being blasted out of existence, including monuments of man's finest handiwork and libraries of man's recorded progress and best thought. These things, once posterity's choicest, rightful heritage, that represented even centuries of development, of saving and of honest toil, are now only rubble and ruin. Truly these are damaging indictments of man's boasted rational behavior.

And, as if these were not indictments enough, we have also to reckon the future effects of this earth-sweeping storm which assuredly carries in its wake enormous impacts and repercussions, the full outcome of which none of us would scarcely dare predict. From these impacts, however, we may be equally sure that no man, woman or child, here or elsewhere, who survives this struggle, can possibly escape. Nor can we imagine an end to its deeper repercussions short of many generations to come. Indeed, recognizing that only the best of younger men these days can meet the requirements of war, we here well realize that of those who are sacrificed without issue, their hereditary, as well as their individual, potentialities are lost to our species forever.

In the face of such world events and of their certain aftermath, what now can we as American scientists, as American naturalists, say regarding future human progress? It ought to be fortunate, for others as well as ourselves, that America is far removed and is made safe from the seething centers of this world holocaust. What might fellow scientists in those centers say, or have a right to say nowadays, even at the very mention of human progress? Our situation, accordingly, affords an opportunity, and it imposes an obligation, to try to view mankind's awful plight with better perspective and more objectivity. This would, of course, be our customary procedure in approaching our usual scientific problems. May not a similar approach, let us say a biological approach, be attempted also on some of our urgent, baffling human problems? To be sure, such efforts would not be new, and various past efforts may have been more effective than we can well realize.

Our symposium to-day was evidently conceived and planned with that hopeful prospect in view. Professor Sinnott's paper will have to do with the promising extraordinary contribution that plants can further make to the material basis of our civilization. All of us can readily sense the import of that theme and are now looking forward with great interest to his discussion. The other paper by Professor Chambers, on the rehabilitation of our fellow scientists in the occupied, war-ravaged countries, presents an obviously human problem, the urgency of which needs no comment. What may best be done in their behalf and how that may promptly be done affects each of us directly and deeply.

My own discussion to-day on "Biology and the Public Mind" may be prefaced as follows: Recognizing civilization as a relatively recent human institution, whose superstructure requires, to maintain stability and growth, a stable foundation, it is suggested that biology can continue to contribute to a better understanding of both the superstructure and the foundation. To that end, it is further apparent that in the future, more than in the past, the added task of biologists will be to clarify accurately in the public mind the biological nature of civilization as essentially an expression of the biological nature of man.

It may here be agreed that the known relatedness of biological nature and human nature, the latter being an extension of the former, has not been adequately exploited, especially in the interests of a better-informed public. Reasons for this seem obvious, for in that endeavor we are at once confronted with certain limitations which are in part traditional, but also they are partly of our own making. Traditionally, they relate to common misconceptions, even in the otherwise informed public mind, about both our scientific method and our scientific system of knowledge and understanding. Science in general has come to be compartmentalized within a given field or fields and barred from other fields. We might recall, in this connection, that naturalists are, by one definition, versed in the natural science of plants and animals—to the exclusion, presumably, of the natural science of man. But such distinctions are not confined to the lay public. We are all familiar with the current view, which seems to be widely reflected in our universities these days, that the departments of human knowledge are in the main mutually exclusive in both their materials and their methods. This obtains especially, it is said, for the natural sciences on the one hand and the humanistic studies on the other hand. Science, accordingly, has not the competence for the solution of human problems. Human qualities are intrinsic qualities that have no essential counterpart in other living things. Scientific tools and procedure have gone far in the analysis of stars and atoms, of vitamins and cells and fruit-flies. These methods evidently are adequate in the quest of nature around us, but they leave the nature of man unapproachable.

Such common misunderstandings of the achievements and capacities and the province of science may come to be even more accentuated after the war, not only in the public mind but perhaps also in our colleges and universities. The tremendous decisive contributions of scientists in the present world conflict have popularly magnified science to its advantage and to its disadvantage. Apparently the final decision in this struggle will depend on superior arms and other equipment more than on greater manpower. The chief task of the sciences still is to help provide as rapidly as possible the most effective means of destruction and the best care of our manpower. Especially through medicine, agriculture, engineering and industry, this is of course being done.

But in the coming aftermath, what may science, especially biological science, contribute toward the even greater task of rebuilding our devastated world and of reestablishing our profoundly disrupted civilization? It is inspiring to contemplate the prospects of a greater abundance of proper food, more widely, equitably distributed; of improved living conditions, improved health and a longer and happier life; of a better quality and perhaps a limited rate of reproduction. These and other advancements are not impossible, and some of these seem fairly assured. But our biological contributions need not, of course, end there.

Indeed, it may here be urged that, along with these splendid prospects toward helping to rebuild and improve this essential superstructure of our civilization, we should surely bear in mind, and help to clarify better in the public mind, the deeper foundation on which that superstructure rests. Otherwise, some of the very factors that enter into the rebuilding of our superstructure may sooner or later again contribute to its disruption.

The identity of that deeper foundation is in last analysis the essential nature of man, whose stability or instability must, in turn, determine the stability or instability of any superstructure built upon it. The conventional view that human nature, stable or not, can not be changed, seems not to have taken into account that human nature unknown may not be the equivalent of human nature known and understood. And we should likely further agree that one of the truly great contributions of our science has been the discovery and elucidation of the biological nature of man, or, for purposes of this discussion, of the relatedness of biological nature and human nature. We should, it seems, regard it a momentous step forward, a turning-point in mankind's study of man, when interest and research came to be focussed less on the nature of things individually and more on things relatedly—when studies on man and the nature of man turned to studies on the related nature of things, including man.

Realization of this related nature of things had to await the discoveries of their common denominators. Such discoveries were indeed a triumph for scientific method and procedure. Where unaided experience had to stop, the scientist was not content to stop. By devising and improving special means to enhance his perceptions of nature, the scientist came to discover unity in nature where only diversity formerly appeared. This discovery of unity, of common denominators and the true relationships of natural phenomena might well merit greater emphasis these days, especially in the interest of a better-informed public. For it may here be agreed that recognition of this unity and corresponding relationships may properly demarcate the modern world from the ancient world. Knowing no common denominators, the ancients saw and emphasized differences in nature's manifestations and so, unable to understand or to control these diversities, they imposed in their gods and goddesses that understanding and control, thereby trying to satisfy their own ignorance and impotence.

These things certainly the public mind may come to know and duly appreciate. Accordingly, a better-informed public may also appreciate that the vast and rapidly advancing knowledge of inanimate and animate nature, which is literally transforming our modern world, owes its strides of progress largely to investigators who, more often than not, were least concerned about the immediate utility of their findings. Instead, their concern was and is better to understand the related nature of things, the beginnings of which obviously go back to the discoveries of units of matter and energy in things inanimate and of units of structure and functions in living things.

Yet how well we realize the present inadequacy of that basic knowledge and how indispensably it must continue to advance, always beyond present frontiers, if human progress is to be maintained. The wide gap that so long has separated inanimate and animate nature assuredly, if slowly, is being closed. The laws of the physical world evidently obtain also in the living world. Properties of the physical basis of life are comparable with properties of other physical systems. The chemical nature of living things from bacteria to man, such as their similar nutrient requirements and enzyme systems, have their simpler counterparts in inanimate catalytic systems.

Thus it would seem that the picture of this related nature of things that is gradually being fashioned may be likened to a mighty spectrum, comprising both inanimate and animate nature, many of the lines of which are still poorly defined, but the existence of which can scarcely be questioned. The lines of that spectrum, however, would represent degrees or levels of organization, from the highest to the lowest in the living world, and downward without break into and throughout the physical world. In like manner, may not the uppermost limits of that so-called spectrum also link biological nature with the essential nature of man? If so, then may not human problems come to be more and more resolvable to biological problems? Hence, a biological approach to human problems is potentially something more than a figurative expression. Its validity inheres in a clear recognition of the biological relatedness of all living things.

Biologists, of course, clearly enough recognize that relatedness, and it is here suggested that it may be better clarified in the public mind. To that end, we are well aware that our science comprises a body of well-established general fact, as well as a reliable method by means of which that body of general fact was established, and by means of which also it is demonstrably true for all, including no less the informed public.

What that body of general fact is, certainly none of us here needs to be reminded. It constitutes the generalizations, the very essentials of our science. Indeed, it unifies our biological sciences into one science. Its generalizations were derived inductively and from them we may reason deductively. These represent the landmarks of progress in biological research and they serve as the guideposts in our elementary instruction. They are, so to speak, the A.B.C.'s of our science, and so in our various specializations they come to be largely taken for granted and perhaps may be unduly neglected. For purposes of this discussion, however, we may particularly note here that some of our most familiar, classical generalizations may most simply and strikingly disclose the essential relatedness of biological and human nature, notably in the interests of a better-informed public.

You will bear with me, therefore, if in briefly recalling this relatedness, I should cite certain of these generalizations that are familiar to every one here, only to point out some of their implications which are also obvious to us but which, it is urged, might well be made more clear and significant to the public. Accordingly I have chosen three classical generalizations that have to do mainly with structural, developmental and functional relatedness among living things, with special reference, of course, to man.

It was during this decade just one hundred years ago that biologists were greatly exercised over the then recent pronouncement of the cell theory. Similarities in the cells of plant and animal tissues were coming to be generally recognized and the viscid contents of cells were being explored. We realize more and more that the importance of those early investigations can scarcely be overestimated. But the point of emphasis for present purposes is that the extension of that theory came to include all living things—plants, animals, microorganisms and man. And so we can say, and the generations to come can say, that the living world is a cellular world, much as the inanimate world is molecular. And we now know that this protoplasmic unit is the unit not only of the structure of living things, but also of their functions, their development, their heredity and their evolution.

Thus all living things, including man himself, in spite of their manifold diversity, have the same unit structure, perform the same elementary functions, begin their individual existence each from a relatively simple, primordial cell, transmit their hereditary factors by means of the same mechanisms, and from the mass upon mass of evidence, all things that live show these unmistakable marks of common relationship by virtue of a common origin.

And it is because of this common relationship that biologists can investigate monkeys or birds or frogs, fish, insects, flowering plants, marine plants, protozoa, bacteria or other organisms, and, if persistently, critically, deeply enough studied, they may come to find there, as others previously have found, not only phenomena that hold true for each of these organisms, or others of their kind, but eventually also some factor that is common to many or all of these and common no less to man himself. Modern studies on the relatedness of gene action and enzyme metabolism strikingly illustrate this.

But organisms are more than a mass of protoplasm or a group of cells; certainly more than only interacting atoms and molecules. Organisms have a history, a developmental, evolutionary history, which comprises both their ontogeny and their phylogeny. This salient fact, perhaps more than any other, distinguishes animate from inanimate nature. And this fact, we all might agree, needs to be taken into account in understanding the relatedness of biological and human nature.

For purposes of this discussion, a few diagrams of the well-known developmental series of animals, including man, are shown here by lantern slide. These diagrams are sufficient to recall that typically each organism, from protozoa to chordates, begins its individual life as an undifferentiated unit of protoplasm, a so-called primordial cell. Thus each living thing takes off at the same level, so to speak, at the primordial cell level. And, as serially arranged, all proceed successively toward correspondingly higher levels of development along a similar course, each to its characteristic, fully differentiated, adult level (Fig. 1).

Divergencies in the courses thus followed are conspicuous and are important, of course, in other connections. But for present purposes, what matters most is the final level at which each line of development ends. The protozoan, beginning as an undifferentiated cell, ends as a single differentiated cell. The coelenterate attains in its ontogeny the gastrula level, but remains essentially a differentiated, gastrula organism. And the successive levels of the flatworm with its third germ layer, of the round worm with its primitive coelome, and of the annelid with its added metamerism and its more highly differentiated organs and organ systems, are all familiar, classical examples. Finally, as also so well known here, the chordate reaches and exceeds all the preceding developmental levels, to attain among mammals, in the epigenetic differentiation of its neural tube, a mammalian brain, a primate brain and lastly the highly differentiated brain of man.

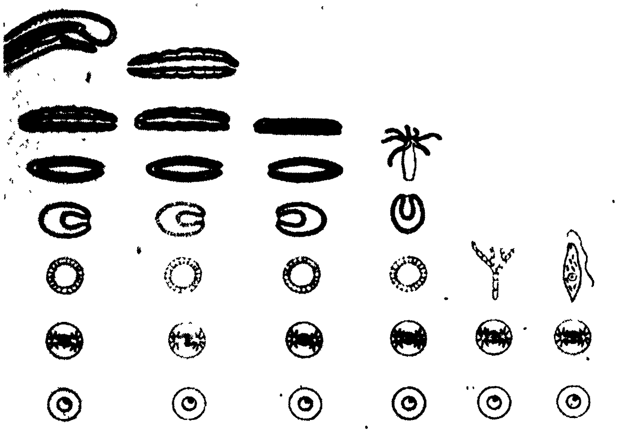

FIG. 1.

If now, allowing for many important omissions, we can observe this degree of similarity to obtain throughout the entire gamut of animal organisms, from protozoa to man, what comparatively can we say regarding the degree of similarity among the members of a single species? Evidently the degree of similarity greatly outweighs the degree of difference. The former, indeed, is the basis for understanding the latter. And would it not appear that too much can be made and likely has been made of the degree of difference between groups of organisms in general, including, for example, the so-called races of men, and so not sufficient emphasis has been given to their degree of similarity? That organisms and groups of organisms differ and that races of men differ is hardly the question. Their variations obviously are always within limits. Is it not, then, the degree of difference that mainly matters? How to determine this and how to account for it remains a major biological problem, indeed many major problems which, especially from genetic evidence, appear now to have been partly solved. But in our efforts to identify and account for differences among men and other organisms, have we not unduly neglected their even more remarkable similarities? It is the latter, of course, that betrays their kinship, their common origin. And evidently it is this kinship, this degree of likeness, especially within our human species, that the public mind may well afford better to know and understand in the coming aftermath.

A further implication which this developmental series makes clear is the extraordinary stability that protoplasm has had evidently throughout the eons of evolutionary history. This, of course, accounts for the degree of similarity among organisms and it merits further brief consideration here in relating biological and human nature. Apparently phylogenetic history has also been essentially epigenetic in character. Each level of development in phylogeny, much as in ontogeny, was built upon preceding levels, always with various modifications, certain abridgements and consequent elisions. This procedure surely obtains no less for the evolutionary history of man. At least the main steps in that history seem to be unmistakable. These main steps have been modified and abridged but, as such, man incorporates them all.

In this sense, then, we may rightly regard human nature as essentially an extension of biological nature. Hence, we bear in our bodily form, and in our elementary functions, and in our reproduction and our heredity certain fundamental characteristics, certain tendencies and demands and desires which have clearly their more primitive counterpart in lower forms of life.

It is now a commonplace to say that man's unique place in nature can be attributed chiefly to the extraordinary superior development of his central nervous system, by means of which not only can he envision a better, healthier, happier civilization than he has ever known but also, it may be, he can thereby determine the means toward gradually realizing that kind of a civilization.

Working toward the fulfilment of that prospect, however, surely man has to bear in mind also that he, like lower forms of life, has a digestive system which demands food and will have food, even eventually at the cost of a deadly and destructive war. The significance of this well-known fact, especially for the public mind to recognize, is that that sort of insatiable, ravenous demand for food recurs in organisms without a central nervous system as well as in organisms having a central nervous system. Evidently, therefore, there is nothing essentially rational about hunger, and it may not be beyond our understanding if the more primitive, nutritive demands of man should at times exceed the bounds of his rational behavior.

In a similar sense, man, like lower forms of life, has differentiated a reproductive system whose natural demands for the procreation of his kind evidently had their origin not with the origin of man, nor with the origin of a central nervous system, but that origin of reproductive demands must have had its inception with the very origin of life itself, for that is one of the fundamental, most primitive, universal characteristics of living things. Is it not probable, therefore, that the surges and upwellings in nature's demands to reproduce have their source for man much deeper than his rational self?

These few familiar examples of the truly profound relatedness of biological and human nature seem to urge that mankind's further study of man, in trying to solve man's individual and social problems, should seek better to ascertain the nature and limitations and degree of stability of his rational behavior as conditioned by his deeper, more primitive, non-rational functions. Since nutritive and reproductive functions, in both their developmental and evolutionary history, are more primitive than neural functions, is it not apparent that their role in conditioned behavior needs also to be duly recognized and well understood? May not such propensities as self and race preservation, common to man and lower organisms and now predominant in our recurring world conflict, spring from depths of our nature of which we are ordinarily unaware? Accordingly, could it be that both our defensive and offensive reactions are deeply rooted in the more primitive, nutritive and reproductive functions, well below our rational functions?

Such considerations serve to remind us that the essential problems of human progress are more than national or international problems. Among us, as naturalists, it may here be agreed that they are, first of all, problems of the human species, of the biological nature of man.

Our discussion thus far has been directed mainly toward's man past, his evolutionary past, as the basis for understanding his essential nature. But man is potentially more than the product of his past. He has aspirations, purposes, ideals which, so it would seem, may be regarded as essentially the more rational extensions of his formerly less rational and non-rational past. His further evolution, *i.e.*, his future progress toward fulfilling his now more rational purposes and ideals, evidently must depend more and more upon his evolving knowledge and understanding. Knowing approximately the laws of nature and the evolution of his own nature, man may now not only look back upon the long and perilous road he has traveled, but, like the mythical Janus, man may also look forward with assurances, such as he in his blind past could never have, to a new and improving world which must increasingly be the product of his own rational creation.

The gradual realization of that kind of an increasingly rational progress would undoubtedly presuppose the continuous, unlimited advancement and diffusion of science, in the broadest and best sense of that term. To that end, then, is it not also apparent that the essential knowledge, the methods and the aims of our science will need to be better, more accurately clarified in the public mind, by improved means both within and outside our educational institutions? For it is the public who, sharing the benefits of human progress, must also share its responsibilities. And among those responsibilities obviously must be not only an intelligent support of science but also the protection of scientific progress, against any one or any group who would divert its proper course or misuse its results to thwart human progress anywhere. Such protection would thus constitute a kind of rear-guard protection of human progress, the vanguard of which will assuredly prove itself to be the unhampered progress of science.

PLANTS AND THE MATERIAL BASIS OF CIVILIZATION

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WHETHER in recent centuries our civilization has really been making progress, or whether, as some insist, we are slowly spiralling downward to barbarism again is a question which historians and humanists and the lapse of time must ultimately decide. But in one aspect of man's life to-day—in his control over matter and energy—he surely has advanced far beyond the levels of the past and still goes forward with no sign of halting. The machine, with all the wealth of goods and services which it has placed at man's command, undeniably represents progress of a sort. We may well doubt its relevancy to those qualities by which the true ascent of man should be measured, and we may question whether his moral stature has yet reached a height where he can be trusted with so much physical power. Here it is that the advance of science touches these human problems so intimately, for the machine, with all it promises both of good and ill, is but an application of that vast fund of knowledge about the universe achieved by chemistry and physics since the eighteenth century. Our society, in a material sense, is built on the physical sciences.

We are less obviously dependent upon biology, though in all these years the biologists were not idle. Since the days of Linnaeus notable advances have been made in our knowledge of plants and animals. A recognition of the significance of protoplasm, of cellular organization, of the mechanism of heredity, of microorganisms, and of the character of metabolic processes generally are a few of the steps in its progress. Despite all this, biology has had a relatively small share in the rapid development of the material basis of civilization. To be sure, our greater knowledge of microorganisms has been of enormous value in the prevention and control of disease, and better understanding of physiology has made of us a healthier and more efficient race. All this is immeasurably significant for the life of man, but of the material things on which this life depends, biology has given him much less than have the physical sciences.

Agriculture, of course, is still the foundation of society, and is concerned with the exploitation of plants and animals for man's benefit; but while applications of the physical sciences have completely changed the face of industry, those of botany and zoology have had much less effect upon agriculture. A farmer from the days of Virgil's "Georgics" would feel at home on an Italian farm to-day, but a craftsman of his time would be quite bewildered in a factory. Much of the increase in productiveness of agriculture in the past three generations has been due to its mechanization rather than to changes in its biological basis. I do not wish to undervalue the contributions of biology here which in the aggregate are very considerable, as in the development of superior animals and plants, in the discovery of more efficient methods of culture, in soil improvement and in other ways; but it is evident that these have been small in comparison to the contributions of the physical sciences to industry.

Such a result is not surprising. The material with which biologists deal is of extraordinary complexity, and much of their efforts thus far have been spent in preliminary reconnaissance. They have had to wait for discoveries to be made in the simpler domains of physics and chemistry before they could apply them to a study of living things. But in recent years the progress of biologists has become accelerated. Working in cooperation with biochemists, biophysicists and other fellow scientists, they have begun to gain a much more fundamental knowledge of the complexities of protoplasm and the organisms which it builds. These organisms are physicochemical machines, at many points intricate beyond our present powers to analyze but nevertheless machines and subject to the same laws which have been found to operate in simpler mechanisms. They possess remarkable capacities for the synthesis of hosts of substances of great value to us, they run by power freely available in immense amounts, and they have an important advantage in that they do not need to be constructed by human effort and artifice but build themselves and even make provision for an ample succession of similar mechanisms. The time has come when our knowledge of these extraordinary living machines will make it possible to use them much more commonly and with far greater efficiency than at present. This employment may well lead to changes in our society so great that they will be comparable to those which followed the industrial revolution itself. Doubtless biology can minister to our time in loftier ways than these, and we never should lose sight of such a vital service. I propose to limit myself here, however, to the purely material level of our civilization and to discuss certain of the possibilities for its enrichment which may grow from the wider use of plants in the service of mankind.

Plants have almost a monopoly on the synthesis of those materials which are of primary importance to life. They hold, so to speak, the basic patents of the organic world. The synthesis of carbohydrates, amino acids and vitamins belongs almost entirely to them, and in their cells are manufactured a host of specific substances which are, or might be, of utmost usefulness. As "the groves were man's first temples," so were they his first chemical laboratories, but laboratories which he could neither understand nor enter. From them he extracted only a few of the more obvious products. It is toward a more complete utilization of the services offered by these remarkable synthesizing mechanisms that man's efforts are now turning.

Most familiar of these services is the production of food. Bread is the staff of life, and carbohydrates have always been the chief source of fuel for the human body. They are the immediate product of the most spectacular synthetic process in plants, photosynthesis. The basic facts about this have long been known, but in recent years many of the details are being filled in. We know that both "light" and "dark" reactions are involved and that they differ in length; that, as shown by "tagged" atoms, it is the oxygen from the water, not from the carbon dioxide, which helps form the carbohydrate molecule; that many specific genes are involved in the development of chlorophyll; that a minimum number of light quanta is concerned for each molecule of product; that photosynthesis and the synthetic activities of some of the bacteria are similar in involving the transfer of combined hydrogen from a donor, such as water or hydrogen sulfide; and even that carbon dioxide may sometimes be built into carbon compounds in protoplasm without the agencies of light or chlorophyll. Such knowledge, if still further enriched and extended, should give us an intimate understanding of how light energy is trapped in the carbohydrate molecule by chlorophyll. This we can not yet repeat in the laboratory; nor if we could would we be able to compete with the cheap labor of sun and soil and leaf which it is the farmer's business to manipulate. But the process in most plants is inefficiently accomplished, and our greater knowledge will surely enable us so to improve these living machines, and to utilize their capacities so completely that an abundance of food may be within the reach of all mankind. Despite all that has been done to this end, much of the world is still hungry; and the spectre of catastrophe which Malthus raised, though pushed aside for a few generations, has never been laid. Agriculture, without a revision of its resources and methods in the light of our new knowledge of plant syntheses, will hardly be able to feed abundantly a much more densely populated world.

The problem of protein foods, those necessary tissue builders without which the physical basis of life itself can not be constructed, is even more difficult. Here, again, plants hold the basic patents, for they alone can bring together carbohydrates and inorganic compounds of nitrogen to form the essential amino acids out of which all proteins must be built. Much more restricted is the invaluable capacity to tap the vast supply of nitrogen in the atmosphere, for this is possible to only a few bacteria; and the most notable among these, the root-tubercle bacteria, are almost confined, in their associations, to one plant family. The nitrogen available to other plants is limited chiefly to ammonium and nitrate compounds. The synthesis in plant tissues of the amino acids, upon which we ultimately depend for all our proteins, is thus subject to many restrictions, with the consequence that the production of a sufficient supply of protein foods becomes the most difficult problem in the nutrition of mankind.

Clearly one of the major tasks for those who would improve the service which plants give to man is to learn more about the manner in which protoplasm builds these compounds which form the physical basis of all life. A modest beginning has already been made toward this end. Amino acid synthesis is not as dramatic a process as photosynthesis, nor so easily recognized. It evidently is not the monopoly of any one organ, however, nor does it need light, for pieces of various tissues, isolated and in the dark, grown by the techniques of tissue culture and provided with sugar and mineral salts, produce protein abundantly. Synthesis seems to take place wherever new protoplasm is being formed, and is perhaps a part of the fundamental assimilatory capacity of all living matter. There are only a score or so of the amino acids, and they are formed through the mediation of enzymes by attachment of one or more ammonium radicles to specific organic acids. How this synthesis is brought about we do not know, but here, again, the "tagging" of atoms by use of radioactive isotopes or "heavy" carbon offers a hopeful method of attack. Plants differ markedly in their ability to synthesize particular amino acids, and these differences can be shown most easily and precisely in bacteria, yeasts, molds and other fungi, plants which have recently contributed much to our knowledge of physiology. In some of these organisms it is now possible to relate particular genetic changes to changes in the character of their amino-acid synthesis.

Practical results are already following studies of the protein metabolism of these lowly plants. One of the most spectacular of recent contributions to nutrition was the discovery that a strain of the yeast species *Torula utilis* is extraordinarily efficient in converting inorganic compounds of nitrogen into protein. This ability is already being utilized on a large scale to produce from the wastes of breweries and distilleries a supply of protein feed for livestock. Plans have also been worked out for the production of proteins for man by yeast, from cheap molasses and ammonia in the West Indies. Indeed, the remarkable versatility of yeasts in the synthesis of a host of useful compounds has led to the present intensive study of the physiology and genetics of these simple plants, which may well find a high place among the living mechanisms which can aid mankind. The yeast which our fathers prized for its mysterious services to bread and beer is a far more potent agent than they ever dreamed.

The nitrogen-fixing bacteria offer further hopeful material. Their secret has not yet been discovered nor the range of their plant associates widened, but enough has been learned of their physiology and of the differences which exist among them to warrant the hope that these differences may be increased to the point where such organisms may attach themselves to other plants than the Leguminosae. Meanwhile, the advantages which these bacteria confer upon this family have given its members a particular significance for man. The soybean, for example, has become a staple of our economy, producing almost everything from vitamins to doorknobs. The proteins made by some of its strains are very similar to those of meat. Protagonists of yeasts and of soys vie with each other as to which can provide the most deceptive and nutritious imitations of T-bone steaks and pâté de foies gras, and vegetable "meats" of many sorts will one day appear on our tables. This does not mean that steers and hogs and capons will no longer form an important part of our diet, but it does show the immense resources, in protein foods of all sorts, which the plant kingdom can provide, and in a form much more direct and economical than from most animals.

Another hopeful method of gaining an abundant supply of proteins is a modern extension of a very old one—the use of the sea. Here untold myriads of minute plants take the abundant inorganic compounds of nitrogen formed from the decay of marine life or coming as outwash from the land, and build them into proteins. These plants, or organisms which feed upon them, ultimately form the food of fish, lobsters, shell-fish and other animals valuable in the nutrition of man and his livestock. It has recently been reported that a man's labor in the California sardine industry will yield several times as much protein as it does in the raising of beef cattle. The careful studies now being made of the sea and its inhabitants show how enormous is the potential supply of protein from this source and how much more fully the synthetic ability of these marine plants might be utilized in the service of man.

Equally important with energy foods and proteins are the vitamins. I need hardly discuss these, for they have become a matter of common knowledge and their name a household word. These substances, too, are produced almost exclusively by plants. The A vitamins are derived entirely from carotenoids, yellow pigments so frequently associated with chlorophyll in green plants. Vitamin C, ascorbic acid, is abundantly produced in plants, which are its source for man. The water-soluble or B vitamins, a notable series of substances, are entirely of plant origin. The unexpected fact has been established that several of these are as essential to plants as they are to animals. Differences in the ability of plants, notably among the lower fungi, to make these vitamins has led to important information with regard to their synthesis. The profound effects of nutrition and other environmental factors upon vitamin production have been explored. Even their genetic basis has been analyzed and the ability to synthesize a specific vitamin has been related to a single gene mutation.

Beyond all this, the essential relation between vitamins and the complex enzyme systems operative in the respiratory processes of both animals and plants have now been established, and provide some of the most intimate glimpses yet gained into the metabolic activities of protoplasm. Studies of vitamin physiology have therefore proven important not only for their original purpose in human nutrition but in the more fundamental task of analyzing the biochemical behavior of protoplasm itself and thus helping to establish the basis for a more efficient employment of protoplasmic mechanisms in man's service.

This better understanding of the nature and synthetic ability of living plant machines will greatly aid in gaining an abundance of these various foods for man; but in other ways, as well, it should also be of utmost service to him. A host of plant products—woods, fibers, rubber, oils, drugs, dyes and many others—are familiar products of plant synthesis and we are far from exhausting their possibilities. The present intensive search for new sources of rubber, quinine, fibers and other materials now urgently needed has shown how numerous are the useful plants not hitherto recognized as such. The efforts of explorers, breeders and physiologists are continually adding to their number and efficiency. Most spectacular, perhaps, in recent days have again been the lower plants, with their production not only of proteins and vitamins but of those wonders of healing, the antibiotics, with penicillin at their head. Economic botany in the broad sense is still in its infancy.

But here an ambitious chemist may interrupt me to point out that many of these useful materials can be synthesized in the laboratory and that man can look forward to a day when he need be dependent no longer, for the myriad materials he needs, upon undirected synthesis in protoplasmic laboratories but can imitate and even improve upon their products in that house of marvels, the chemical laboratory. Ours is the age of chemistry. Chemurgy, the modern alchemy, devotes itself to just this synthesis of useful goods. It points to the manufacture of vitamins on a commercial scale; to the tremendous production of synthetic rubber, and to a multitude of other examples which are familiar to every one. But chemurgy goes much further than merely imitating plant products. It has provided us with scores of new ones. Synthetic fibers have revolutionized the textile industry. The use of plastics for almost every purpose leads some already to speak of the post-war world as the age of plastics. Synthetic drugs, like the sulfa group, have opened up new possibilities of healing. Give a chemist alcohol or cellulose or petroleum and he can make almost anything under the sun! What wonder that enthusiastic chemurgists believe our society will be made over by their art; that self-sufficiency will replace impoverishment for "have-not" peoples, and that international trade will no longer be concerned with raw materials.

But welcome as are the tremendous possibilities of chemistry for man's service, we should not forget two fundamental advantages which syntheses in plants have over those in the laboratory, advantages which will keep the material basis of our civilization still anchored firmly on the vegetable kingdom.

First, even with these plant products which can be made synthetically, this is in many cases uneconomical. Plants have, so to speak, such cheap power, raw materials and labor that they can defy competition from industries less fortunately situated, so that for most plant products there seems little likelihood of replacement by synthetic substitutes. Indeed, chemical treatment of such products often so enhances their usefulness as to result in a much greater demand for them. Perfection of methods of wood treatment by which satisfactory pulp can be made from a wider variety of species than before, and of other techniques which produce much improved modifications such as plywood, plastic wood and impregnated wood, have enormously increased the usefulness of wood in our civilization, and made it of critical importance in the war. Not many years ago wood was regarded as a primitive sort of material, destined to be superseded by products better adapted to our mechanized society, and its return to a place of major importance is indicative of what may happen to other plant products.

Second, and more important, even where entirely new poducts are made, like rayon and the plastics, these must use as raw materials either the basic carbohydrates or a modification of them. Plants alone can unite carbon dioxide and water, and add nitrogen to them. These feats the cleverest chemist has not yet duplicated nor is he likely to do so, at least on a commercial scale. He must always come back to green plants.

The greatly increased demand for these basic products of plant synthesis, however, makes the growth of chemurgy highly significant to students of plant production. They must evidently devote their efforts less to the employment of plants for the synthesis of highly specialized materials and more for the mass production of raw stuffs needed by the chemist. Emphasis must be placed more on quantity than on quality. New plant sources must be explored. Surely it should not be necessary to use the grain harvest from 10,000,000 acres and much of the sugar crop, as we are now doing, to produce industrial alcohol. The demand for this material, especially acute now from its use in the making of rubber and explosives, will doubtless continue high during peace time because of the growing use of alcohol in industry. Here lies a great future for making alcohol from sawdust and other wood wastes, on which such a hopeful beginning has already been made. The Scholler process, for example, is said to yield fifty gallons of alcohol from a ton of wood waste.[1] Here again, forests with their greater yield of plant material per acre per year, take on increased importance. More attention will also be given to the development of agricultural crops especially adapted to the requirements of industrial synthesis. The great increase in those chemical techniques which are certain to yield such a large part of the materials needed by the society of the future therefore does not mean that we shall depend less on plants but rather that we shall use their synthetic capacity even more, though often in different ways. Agriculture will continue to be the primary profession of mankind.

In addition to food and industrial materials there is a third way in which plants seem destined to contribute mightily to the material basis of our civilization. Society to-day rests upon machine industry, and machines need power. Coal and oil, the great sources of power, are reservoirs of the energy-rich products of photosynthesis in ages past. How long our supply of these invaluable materials will last is a much debated question. Some authorities say a shortage will begin to be felt in a decade or two; others that we need not worry for a few centuries more. However this may be, one fact is clear—this source of power is limited in amount and is being used up at an ever-increasing rate. War has shown how seriously a fuel shortage affects every aspect of our lives. Some day, assuredly, we shall have to get part or all of our power from other sources than organic matter in the earth's crust, and it is time that we began to explore their possibilities. Water power, wind, tides and atomic energy are either limited in amount or efficiency, or impossible to develop on a large scale. The direct fixation of solar energy by green plants in carbohydrate synthesis is still the most promising method of producing

[1] H. Harvey, *Science News Letter*, 45: 218–219, 1944.

materials which can be used as sources of power, and products of photosynthesis in living plants may before many years assume an importance in this respect equal to the products of this process in plants of past ages.

The total energy available from the sun is enormous—about 7.47×10^{23} calories per year,[2] roughly equal to that liberated by the burning of 1,000 tons of coal over every acre of the earth's surface. Only a very small fraction of this is now fixed by green plants. It is estimated[3] that a field of vigorously growing corn plants uses in photosynthesis only about 1.6 per cent. of the light which falls upon it, and an acre of apple trees only 1.0 per cent. For the entire earth's surface, calculation shows that only .18 per cent. of the solar energy is fixed by plants.[2] Despite this inefficiency the total production of material is enormous. Some recent figures[2] indicate that land plants make about twenty billion tons of organic carbon per year. Another authority[4] estimates the amount as about thirty billion tons. Recent figures by him give the total world consumption of petroleum as the equivalent of 2,940,000,000 tons of carbon per year, so that the fixation of carbon by plants is equivalent to from six to ten times this amount.

These figures doubtless have a wide margin of error, but they agree in showing that the yearly amount of sun energy locked up in terrestrial plant materials is well in excess of the amount used to power our machines. Some of this product, of course, must be used as food for ourselves, our domestic animals and other living organisms. Some must be reserved for industrial purposes. Much would be lost in conversion to an available form. The remainder, theoretically available for fuel, is therefore probably not very greatly in excess of our fuel requirements. A well-planned economy should look forward to means for increasing this photosynthetic product and making as much as possible of it available to man against the day when coal and oil grow scarce.

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[2] G. Riley, *American Scientist*, 32: 129–134, 1944. (References here to the papers of Noddack and Schroeder in this field.)

[3] E. N. Transeau, H. C. Sampson and L. H. Tiffany, "Textbook of Botany," p. 184. New York: Harper and Bros., 1940.

[4] E. Berl, *Science*, 99: 309–312, 1944.

Several methods to this end suggest themselves. The most obvious is to increase agricultural production, almost all of which now goes for other purposes than fuel. Some increase can come from the development of more efficient crop plants, attainment of optimum growth conditions and elimination of losses from insects and fungi. More land can be brought under cultivation, especially in those wide areas of the tropics where conditions for plant growth are so favorable. More especially, crops can be grown which yield maximum amounts of plant material per acre, like sugar-cane and sweet potatoes, rather than many of our present staples.

A second line of attack is to secure a more complete utilization of the great body of plant products which now grow without cultivation, notably forest trees and other wild plants. Much more than half of the total terrestrial plant production is by forests, and per unit of area, annual production of organic material here is nearly half again as high as from cultivated land.[3] Forestry can doubtless increase this yield, and much could serve as fuel if efficient methods of conversion were developed. I have already mentioned the production of alcohol from sawdust. Another promising method has recently been proposed[4] for converting cellulose with relatively little energy loss into a product somewhat like petroleum. Not only wood can be used by this process but all cellulose wastes, which in the aggregate are very great, both in forests and on cultivated areas. These ordinarily decay, and by making them into fuel we should simply divert to our own uses the vast amount of energy now dissipated in the metabolism of the microorganisms which cause this decay.

A third source of power from plant syntheses, perhaps ultimately the most hopeful of all, is in the sea. Measurements of photosynthesis by algae of the marine plankton is difficult, but recent estimates[2] indicate that the production of organic carbon by these plants is in the vicinity of 126 billion tons per year. This is more than six times the 20 billion tons already mentioned as a good estimate of production on land. It represents a yield of about 340 tons per square kilometer, more than twice the average of 160 tons for cultivated land. These are certainly noteworthy figures, for they show the tremendous photosynthetic capacity of marine plants. If we could tap effectively this enormous reserve, our worries as to carbohydrate production, whether for fuel or other purposes, would be over. Methods toward this end are hardly beyond the speculative stage, but it is clearly a major field of research for both the oceanographer and the botanist.

So through the years grows man's dependence on those living machines, the cells of plants. Despite perfection of mechanical techniques we shall evidently become more reliant, not less, upon the synthetic abilities of plants for the food we eat, the industrial products we require, and ultimately the fuel that shall power our mechanized civilization. The more extensive and efficient use of these protoplasmic mechanisms will require a much more intimate knowledge of their character and activities than we now possess. To provide this knowledge is the most urgent demand ever made upon the plant sciences, and places upon every botanist, and his collaborators in kindred fields, an important share in the responsibility for extending that command over nature upon which society depends. Protoplasm is the most intricate of mechanisms and its investigation is beset with endless difficulties, but very real progress has been made, and will continue, on many fronts. The unique capacity for photosynthesis, upon which rests so much of the significance of plants for man, must be explored more intensively. The establishment of the Kettering Foundation for work in this field is evidence of the importance which it holds in the eyes of enlightened industrialists. Similar studies must be extended into the equally notable ability of plants to make simple nitrogenous compounds. The whole field of microbiology, which offers so many hopeful approaches to fundamental problems, must be carried far along the road it has so brilliantly opened. A study of all the so-called "growth substances," which has proven so stimulating to plant physiology and offers so many possibilities for practical application, must be carried much further. Other methods for the control of plant growth—hydroponics, vernalization, photoperiodism and more, unknown a few years ago—must be more widely extended. Genetics, which offers so much for plant improvement and has recently harnessed the tremendous stimulus of hybrid vigor in crop production, has many new paths to open, especially the fundamental problem of the mechanism of gene action in development. The plant explorer should by no means be forgotten, for surely there are hundreds of species, particularly in the wide tropical forests, which could be of great service to man. Those oceanographers who are opening up the tremendous domain of the life and activities of marine plants and animals may make a contribution beyond all the rest, and should be vigorously supported. Promising techniques of all sorts should be utilized. The beautiful method of exploring the inner recesses of metabolism by following the course of particular atoms which have been "tagged" by the use of radioactive or of "heavy" isotopes holds tremendous promise for us. Techniques for the sterile culture of bits of plant tissue opens many possibilities. Measurements of bioelectric potentials may give clues to be found in no other way. Indeed, whoever studies plants as living organisms and by whatever methods may make important contributions to the great problem which we face.

In an endeavor to improve plants for the service of man we should be encouraged by the fact, so often observed, that despite age-long evolutionary advances, plants are in many ways still notably limited and inefficient mechanisms, and that their possibilities for improvement are far greater than have ever been realized in nature. One of our tasks is the stimulating one of directing their further evolution to our advantage and thus sharing, in small measure, in the great process of creation.

Difficulties in all these problems lie not in the absence of promising lines of attack but in the lack of men trained for the task. Well may we say that the harvest indeed is plenteous but the laborers are few. Laboratories supported by government, by industry and by our universities continue to multiply, but it becomes increasingly hard to staff them with properly qualified men. The great prestige of chemistry has in many cases persuaded administrators that chemical training alone is enough; but these protoplasmic mechanisms with which we work are of a different and more intricate sort than any machines of man's device, and the chemist is too often prone to a naive underestimation of their complexity. To investigate them successfully and to harness their powers to the service of man requires not only the arduous discipline of the physical sciences but a familiar acquaintance with that strange phenomenon which we call life. This difficult task must command fruitful collaboration from many sister sciences, but it is primarily the responsibility of plant biologists. To attract into this service a host of able students and to give them the broad yet thorough training which their profession demands will require from the leaders of the plant sciences a degree of vision and of statesmanship which transcends the narrow and technical view of this field which is held too often by the uninformed. The intensive employment of plants to provide an ever wider material basis for our civilization is one of the major tasks of our time, and here botanical science faces an opportunity for highest service to the life of man.

REHABILITATION OF THE BIOLOGICAL SCIENCES IN THE POST-WAR PERIOD

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NEVER before has our organized civilization been brought face to face with such determining destinies, constructive and destructive, as are rampant to-day.

We all appreciate problems which confront us here at home. It is my purpose to show that concomitant with these are world problems vitally interrelated with ours. They have a common solution.

What is the scientist to do about this? Should he remain aloof from the social trends about him, believing that he can best serve by remaining in the seclusion of his laboratories? We know that, in his laboratory, he has developed a method with which he appraises his research, the scientific method, an objectivity of logical deductions based on observed facts. Is it not time that such objectivity be brought to the attention for use by our politicians and those who aspire to world making? But, in order that this scientific method be fully expressed, it is essential that scientific endeavor be free everywhere. Science takes no cognizance of national frontiers. Our best achievements require the sustained thinking of many minds in many countries. Hence, does it not appear that we, as scientists, can serve, not only as sponsors of the scientific method, but also as cooperators toward international unity?

Of immediate concern is Europe. In plans for the rehabilitation of her scientific and cultural institutions, there must be taken into account, not only the ravages of war, but also the building up of forces which are destructive to unity in science. Such debasing forces will have accomplished their aim, unless those who stand for the ideals of true science, take positive action in securing mutual cooperative fellowship, closer than ever before.

All agree that Europe must be free to work out her own salvation, but self-sufficiency in science is fictitious. A science tainted by a prejudicial attitude in one region of the earth will mislead many elsewhere. If we are to build up a healthy science here we must ensure intellectual freedom with opportunity and means for complete freedom of research, teaching and intercommunication everywhere. Science is a search for truth and she suffers if prostituted anywhere.

America will emerge from this war more important than ever before as a power in this world. Can we shirk our consequent responsibilities? The common man in Europe has long been impressed by America. To him America has been the haven of the oppressed and untainted with the evils of aggrandizement and imperialism. To us these values are worth many armies. Armies we shall have, and political power we shall have, but how much are we to implement these with intellectual and spiritual power?

One of the tragic consequences of war is the setback inflicted on progress in the basic sciences. On the other hand, a tremendous impetus has been given to the investigation of applying the bits of knowledge already acquired. This has, of necessity, disturbed the proper equilibrium between the basic and applied sciences. There has also been a shift of public interest and support to the application of the sciences rather than to the basic sciences which make the application possible!

Already, governments are beginning to consider ways and means of organizing scientific endeavor toward post-war security. In a recent message (*Nature,* editorial, 15/1/44) to the India Science Congress Association, Prime Minister Churchill said: "It is the great tragedy of our time that the fruits of science should, by a monstrous perversion, have been turned on so vast a scale, to evil ends. But that is no fault of science. Science has given to this generation the means of unlimited disaster or of unlimited progress. When this war is won we shall have averted disaster. There will remain the greater task of directing knowledge lastingly towards the purposes of peace and human good. In this task the scientists of the world, united by the bond of a single purpose, which overtides all bounds of race and language, can play a leading and inspiring part.'' With reasonable effort the aspirations of this inspiring statement are bound to come true. But the issue must not be confused by restricting endeavor to utilitarianism. The application of science to industrial problems is popular. It won us the war. For this reason, Churchill's statement that ''the task of directing knowledge lastingly toward the purposes of peace and good will'' may be popularly misread unless the full significance of the term ''lastingly'' be realized. Then only can we hold that ''science is pursued for the sake of knowledge of what eternally exists and not what comes for a moment into existence and then perishes.'' (Plato, Bk. III.)

Adequate provision must be made for the pursuit of knowledge for its own sake. We must strive to the end that scientific ideals be followed without hindrance and opportunities be open throughout the world. The solution of our economic, political and ethical problems requires the application to them of the scientific method. Out of this follows international cooperation, the value of which is twofold. It feeds science and accelerates it. It also is the most effective and, thus far, the least used tool we have for mutual understanding and good-will. Our active participation in the rehabilitation of post-war Europe presents an immediate challenge of implementing our aims toward scientific coordination in the post-war world which will be real and lasting.

The National Academy of Sciences and the National Research Council, both intended originally for wartime duties, have been eminently useful as permanent peacetime measures. The National Research Council possesses a Division of Foreign Relations which was initiated when the council was established in 1916. It is a member of several international unions, of which one is the International Union of Biological Sciences. Many of its more active organizations deal with subjects such as geodesy, seismology, oceanography, which, by their nature, require continual international intercourse.

Of considerable interest is the contact of the National Research Council with the International Council of Scientific Unions which advises the League of Nations concerning scientific matters. In July, 1939, a meeting of the International Council of Scientific Unions took place in the Secretariat of the League of Nations at Geneva. In the Report of the National Academy of Sciences for 1939–1940 the plan proposed was presented as follows: "The plan was to consider the progress, interconnections and new directions of advance in the mechanical, physical, chemical and biological sciences with a view to promote the development of the scientific world picture. As a basis for this study, the countries adhering to the council were requested to contribute résumés of the status of scientific research in these several countries." The war put an end to further action which might have initiated a new era in science!

In the Report of the National Academy of Sciences for 1942–3 the ruthlessness of the present war is deplored and, by contrast, an incident of 1813 is related which occurred "during the war between France and England when Sir Humphry Davy visited Paris and was awarded a gold medal of the Académie des Sciences, and elected corresponding member. Such amenities have long since vanished." But should we desire such amenities to persist? That was the time when war was regarded as a chivalrous pastime, a gentleman's profession! Since then, its masks have been successively ripped off and war stands out to-day in its stark reality of unmitigated brutality and wickedness. Shorn of every vestige of decency it can not long survive!

During the war period, attention has been largely devoted to Inter-American activities. The International

Union of Chemistry is organizing a Hemisphere Branch of the union for the purpose of "bringing together chemists of the American Republics. Students of chemistry are being welcomed and trained in the United States." In 1942 the governing board of the Pan-American Union set up an Inter-American Institute of Agricultural Sciences. This was supported by a grant-in-aid from the Coordinator of Inter-American affairs. The Division of Biology and Agriculture of the National Research Council had long advocated the establishment of such an institute. A project also has been started to provide scientists in the other American republics with copies in microfilm of scientific literature available in this country.

Somewhat further afield have been the activities of the American Astronomical Society which, with financial aid from the National Research Council, "has distributed to astronomers in Sweden, Finland, Great Britain, the Union of Soviet Socialist Republics, and Switzerland, information regarding advances in a monthly news letter. Further distribution through embassies and foreign astronomers has brought favorable comment from Australia, Argentina, Mexico, Turkey and other countries."

The Department of State is actively participating in the furtherance of international good-will. The activities of the Division of Cultural Cooperation, established in 1938, are presented in a 71-page pamphlet, "The Cultural Cooperation Program," 1938–43 (obtainable from the Superintendent of Documents, Government Printing Office, Washington, D. C.). During the period of 1938–41 the program was focussed on the Western Hemisphere. This has included travel and study grants; the exchange of professors and books; assistance to U. S. cultural centers, such as libraries, institutes and schools; and the distribution of informational motion pictures. In 1942 the scope of the program was enlarged to the extent of urging "a vigorous development of cultural relations between the people of the United States and other free peoples of the world, for the purpose of fostering helpful international relations on a basis of mutual understanding and appreciation.'' The plan includes ''interchanges in all fields of the arts, sciences, technology, letters and education, and throughout the entire ramp of economic and social life.'' ''The interchanges should serve to promote human welfare and help to preserve intellectual and cultural freedom.''

Encouraged by the scope of this program, a group at the Marine Biological Laboratory at Woods Hole invited a representative of the Department of State to address the scientific community at Woods Hole. Mr. G. Howland Shaw, Assistant Secretary of State, sent, as his representative, Dr. Ralph E. Turner. As a result of stimulating sessions with him a memorandum was prepared expressing the interest of the biologists in participating with the State Department in efforts toward rehabilitation of the basic biological sciences during the post-war period. Briefly stated, the memorandum recommends closer cooperation than hitherto among scientists throughout the world. The need for rehabilitation as a result of war devastation is presented as an opportunity for furthering such cooperation. Suggestions are offered (1) of increased support toward international scientific congresses taking into consideration the inestimable value of frequent personal contacts; (2) of the establishment of representatives of the basic sciences in governmental embassies to enhance close coordination of scientific investigations; (3) of prompt rehabilitation of scholars of the basic sciences with needed laboratory and library equipment; (4) of the establishment of short- and long-term exchange fellowships and professorships.

A memorandum, similar in scope, has been recently and independently prepared by Joseph Needham at the University of Cambridge and was sent to our National Research Council for consideration. It is entitled ''Memorandum on an International Science Cooperation Service.''

In the last published Report of the National Academy of Sciences for 1942–43, the extent to which international matters have been considered, aside from those already referred to here, is shown by the following: In a conference held on August 7, 1942, between one of the secretaries of the Royal Society of London and the chairman of the Division of Foreign Relations, it was agreed that "after the war opportune cooperative understandings with learned societies of previously hostile nations might be a better way of restoring mutual friendship and respect than an attempt, now, on our part alone, to fix a definite policy of collaboration."

However, early in 1944 the Foreign Relations Committee of the National Research Council organized plans for a conference on "Problems of Restoring International Scientific and Scholarly Cooperation." The topics being considered are the exchange of scientific literature, restoration of libraries and resumption of international activities. Plans are also being prepared as to the means of obtaining information regarding needs of scientific workers, exchange of scientific personnel and the relation of present international scientific organizations to meet a United Nations' organization, as may be created. A notable feature of the program is enlisting the interest of the Department of State, with reference to assistance in participation of specific projects of research and publication, to congresses held in the United States and to attendance of Americans in international congresses in other countries.

It is stimulating to realize that the immediate need of post-war restoration is serving to institute, more intimately than hitherto, long-range cooperation among workers in the basic sciences of all freedom-loving countries throughout the world.

The preparation of such a project requires action on the part of every one interested. At least two steps should be taken: (1) contacts should be made to coordinate the activities of foreign and domestic relief committees, which are engaged in plans for the rehabilitation of educational facilities in the war-stricken countries. In such efforts notable assistance is already being given by the Rockefeller Foundation to foreign scientists and to scientific institutions abroad. The Royal Society of London is also engaged in similar activities. (2) By means of Needham's and the Woods Hole Memoranda, or a combination or revision of both, there should be secured at once the active collaboration of all interested individuals and institutions both in North and South America and in other freedom-loving countries overseas, which have been least affected by the material damages of the war.

Ideas emanate from the least expected places. The broader the source and the wider the range of proposed ideas, the more likely will be the chances of preparing a permanently working plan.

A comity of nations depends upon mutual respect for the differences which exist among them. There also exist threads of similarities, a chief one of which is the universal appreciation of science and the scientific method. This provides one of the surest approaches to mutual understanding and good-will.

THE SPADEFOOT TOADS IN OKLAHOMA WITH A SUMMARY OF OUR KNOWLEDGE OF THE GROUP. II

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THE BREEDING PATTERNS OF SPADEFOOTS

Those who have studied at first hand the breeding habits of the frogs and toads of the moister regions of the country only have usually found that most of them manifest several tendencies in common, although of course differing specifically in details of behavior. One of these common tendencies is to have a rather definite season during which all breeding of any one species takes place. Another is the tendency for this breeding season to begin early in the spring (as in various hylids and the leopard frogs), although some (as the American toad in the East, and the bullfrog) wait till later. While most of these species may be stimulated by spring rainfall, almost none are dependent upon this for the initiation of breeding behavior and, with limited exceptions, none breed (either with or without more rainfall) after the breeding season has passed. Because of these trends, making up what I choose to call a breeding pattern, it has been widely assumed that all our frogs and toads have a breeding season, modified to some extent by rainfall and more by latitude (or factors limited by this).

My studies with the Oklahoma forms have convinced me that not all North American frogs and toads manifest the same breeding pattern (Bragg and Smith, 1942). In Oklahoma there are two principal patterns, one characteristic of those species especially adapted to drier habitats and manifested especially by those limited to the grasslands or other more xeric environments; the other shown by those organisms which inhabit moister environments (woodlands, savannahs, etc.), whether restricted to these habitats or not. The prairie-limited species, and others living in woodlands as well as in the prairie, commonly do not have a breeding season at all except in so far as the whole period from early spring to early fall may be considered to be one. For convenience, I propose to call the breeding pattern characterized by a breeding season little influenced by rain the *mesic pattern,* that characterized by no breeding season with all breeding initiated by rainfall, the *xeric pattern.* All the eastern species of *Rana, Bufo* and Hylidae tend to have the mesic pattern. In Oklahoma, *Rana catesbeiana, R. a. areolata, R. a. circulosa, R. clamitans, Bufo a. americanus, B. woodhousii fowleri, Hyla v. versicolor* and *Pseudacris streckeri* are among those exhibiting the mesic pattern. On the other hand, *Bufo cognatus, B. compactilis, B. insidior, Pseudacris clarkii, Ps. triseriata, Microhyla olivacea* and all species of spadefoots have the xeric pattern. *B. woodhousii woodhousii* and the common leopard frog (somewhat doubtfully referred to *Rana sphenocephala* Cope in all my publications) are intermediate, both breeding early in the springtime regardless of rain but also breeding at any time to early fall with any considerable rains which fall. The tadpoles of the leopard frog have been found at Norman, Oklahoma, in January developing from eggs laid the previous fall.

As related to the spadefoot toads, the xeric pattern is most interesting; for nowhere else is it shown in so clear-cut and unequivocal manner. All species so far studied, regardless of their normal environment, are alike in the following: (1) *There is no definite breeding season.* Congresses of these animals may occur at any time from early spring to mid-September whenever conditions are right. Or, in any given area, spadefoots may not breed at all during a year (or several years) if conditions fail to be suitable during this time. Trowbridge and Trowbridge (1937) seemed to have evidence of a breeding season in *S. bombifrons,* but Bragg and Smith (1942) have shown that this was not the case. (2) *Spadefoots usually breed only during or after rains.* Only one exception is known—Linsdale (1938) reported breeding activities of *S. hammondii* in Nevada when a mountain stream overflowed its banks in a dry area. (3) *Spadefoots breed only (or almost exclusively) in temporary water.* (4) *Spadefoots of both sexes are greatly stimulated by the calls of their own species and tend to migrate toward places where others are calling.* Hence, the usual spadefoot chorus in a given pool is a loud and very noisy one. (5) *All male spadefoots have loud, rather harsh voices with great carrying power.* A large chorus of *S. bombifrons, S. hammondii* or *S. couchii* can be heard for at least two miles on a still night and much farther when carried downwind. The call of the individual *S. hurterii* appears to be not so loud, but I have detected a chorus of not more than a dozen individuals from a measured distance of 1½ miles against a breeze and over two low hills in woodland. All who have reported on *S. h. holbrookii* describe its call as very loud and harsh. (6) *Spadefoot breeding is inhibited by low temperature,* but the exact conditions are unknown except for *S. bombifrons.* This form emerges to breed in numbers at an air temperature above about 11° C. (Bragg and Smith, 1942), although great stimulation by rainfall when in conflict with inhibition by cold varies this point slightly, so that a few males may call at and just below 9° C. Emergence of *S. h. holbrookii* was thought by Ball (1936) to be influenced by ground temperature. The conditions with *S. hurterii* are somewhat uncertain. In the spring of 1942, they did not enter known breeding pools and could not be found out feeding all through early April, after rains which made this the wettest April in central Oklahoma for many years and at temperatures varying between 9° and 15° C. But on April 24, a small breeding congress developed during a rain which totaled less than many which has occurred earlier and at lower temperatures. It is possible that an accumulation of rainfall, such as suggested by Trowbridge and Trowbridge (1937) for *S. bombifrons,* may be necessary for *S. hurterii* (and perhaps also for *S. h.*

holbrookii). Should this prove to be the case, then of course the temperatures observed in the spring of 1942 would have questionable significance. The effect of temperature on the breeding of *S. couchii* is apparently wholly unknown. It so happens that I have had opportunity to observe them only in late spring and summer when nothing could be learned about this. (7) *Spadefoot embryos and larvae have an intrinsically high developmental rate.* This has been shown particularly for *S. bombifrons* by Trowbridge and Trowbridge (1937) and by M. S. Trowbridge (1941). For *S. h. holbrookii* it has been mentioned by numerous investigators; for *S. couchii* by Ortenburger and Ortenburger (1936); for *S. hammondii* by Bragg (1941c) and for *S. hurterii* by Bragg (1943). (8) *Developmental rates vary greatly with temperature under otherwise comparable conditions.* Spadefoot tadpoles (at least of all the western species which I have studied) can withstand very much higher water temperatures (and greater fluctuations) than usual with Amphibia. I have found them living normally in prairie pools at water temperatures between 39° and 40° C.; temperatures, moreover, higher than those which have killed tadpoles of *Pseudacris streckeri* and *Bufo a. americanus* in other pools in the same region. Under these conditions, tadpoles of the same age within one pool have varied considerably in size although there is an interspecific difference in degree of such variation, especially in *S. hammondii* (Bragg, 1941c). (9) *The tendency of the tadpoles toward carnivorous habits and cannibalism is marked.*

The type of habit-patterns exhibited by spadefoots, irrespective of the ecological or geographic distribution now shown, suggests strongly adaptation to drier habitats than some of them now occupy. In fact, this alone is enough to indicate strongly an origin of the group in deserts or drier types of grassland from which some species have now spread to moister regions such as the eastern woodlands. This hypothesis is also in accord with the fossorial and almost completely nocturnal habits of the adults and it will explain the marked difference in behavior of *S. h. holbrookii* from all other Salientia of the East, a fact which has always occasioned much interest and often no little surprise when first observed.

If we keep this idea in mind while we examine the interspecific or intergroup differences among species of *Scaphiopus,* further interesting interpretations emerge. The facts upon which these interpretations rest are as follows:

(1) In relation to breeding after rain, *S. h. holbrookii* and *S. hurterii* are greatly stimulated by large amounts of rainfall; not, apparently, so much by its rate of fall. Practically all accounts of the former species speak of the rain which stimulated the observed breeding congresses as *heavy* rather than as *violent* (see Ball, 1936). In regard to *S. hurterii,* it is of interest to note that I have watched the area in central Oklahoma where this species was first reported by Smith and Leonard (1934) for six consecutive years without once finding evidence of their breeding until the record-breaking rains of 1941 (Bragg, 1942). During that spring, however, several congresses developed both here and elsewhere in eastern Oklahoma and tadpoles went through metamorphosis (Bragg, 1943). But, in the spring of 1942, in the same area, these animals did not breed in numbers, although a few males called from one pool during a heavy rain one evening.

On the other hand, *S. bombifrons, S. hammondii* and *S. couchii* all may breed successfully after as little as one half inch of rain, provided its rate of fall is exceptionally fast. This has been most clearly demonstrated in *S. bombifrons* by Bragg and Smith (1942) who also show that this species does not always breed after non-violent rains of as much as two inches when all other conditions are favorable (see also Bragg, 1941c). It seems clear, therefore, that violence of storms is the important factor in this group of species whereas amount of rain is secondary, the exact reverse of conditions in the first-mentioned group. A very violent storm (whether also heavy or not) may overcome some of the inhibition of low temperatures or the tendency toward completely nocturnal habits, in *S. bombifrons* at least, but there is no case on record, so far as I can find, of *S. h. holbrookii* or *S. hurterii* breeding in daylight.

(2) Breeding sites often seem to be selected differently among the various species. I am informed by Dr. A. I. Ortenburger (see Ortenburger, 1926) that in Arizona he observed *S. hammondii* in a small pool only about 100 yards from *S. couchii* in a larger one of a different type. He could not remember with certainty that there was no mixing of species, but he is sure that there was a very great preponderance of the one in the first pool and of the other in the second. Similarly, in New Mexico (Bragg, 1941c) I noted that some pools developed congresses of either *S. bombifrons* alone or *S. hammondii* alone, whereas others contained a mixture of the two. It is interesting to note that where individuals of both species were present, they seemed to be in approximately equal numbers. Only rarely did I find a pool mostly of the one with only a few of the other. Similarly, Dr. Charles C. Smith and I have noted in southwestern Oklahoma that *S. couchii* more often breeds in the same pools as *Bufo compactilis* than in those pools also containing *S. bombifrons,* although we have seen these spadefoot species together on several occasions. The evidence, so far as it goes, indicates that depth of water may be one factor in this situation, although we need more observations on this specific point. From several years' experience with an aggregate of several hundreds of breeding congresses I have a definite impression that *S. couchii, S. hammondii* and *S. hurterii* commonly breed in more shallow water than *S. bombifrons. S. multiplicatus* at least sometimes uses shallow water (Taylor, 1942) and *S. intermontanus* also uses shallow pools, even if the water be brackish (Tanner, 1939).

There are two complicating factors here, however, which must not be overlooked: (a) all spadefoots which I have observed manifest a marked tendency to use any temporary water available and all those which usually are found in deep water may breed in shallow water if deep water is not present in the immediate vicinity; and (b) the observed segregation in pools may be only the result of what type of pool is found by the first emerging spadefoot of any one species because of the marked attraction to the calls of its own kind. These are undoubtedly factors in a complicated situation which only further experience can finally clear up.

(3) The tadpoles as well as adults of *Scaphiopus* are interesting in that they differ subgenerically in general size. Those of all species of Spea are larger and develop into at most medium-sized adults. On the other hand, those of subgenus Scaphiopus are much smaller but develop into larger adults. This is shown by summarizing lengths of adults and larvae as given in the literature cited and from my own measurements. Calculation shows that in all species of Spea the tadpoles are approximately as long in total length as the adults, whereas in Scaphiopus they are not nearly so long, either relatively or absolutely. Adults of Spea are about 55 mm in length, their tadpoles 59 (disregarding as unimportant to the point being made here the considerable variation among individuals). The same figures in Scaphiopus are 65 and 25, respectively. Furthermore, there is not nearly so much interspecific as subgeneric variation, as shown by the following approximate figures of length in mm of adults and larvae (adult figures given first): *bombifrons,* 55 and 65; *hammondii,* 53 and 68; *intermontanus,* 55 and 51; *multiplicatus,* 55 and 51; *h. holbrookii,* 61 and 28; *hurterii,* 65 and 23; *couchii,* 70 and 25.

(4) Another difference in tadpoles among certain species may or may not have significance. Those of *S. h. holbrookii* sometimes form schools in which there seem to be cannibalistic tendencies manifest (Ball, 1936). Those of *S. hurterii* also may form schools, but no cannibalism has been noted. In the latter case the phenomenon is clearly social in character as observed regularly in a single pool when the tadpoles approached metamorpho-

sis. (For details see Bragg, 1943.) So far as I can determine, such behavior has not been observed in any species in Spea.

(5) Behavior during and just after metamorphosis also varies among species. This has been noted specifically for *S. bombifrons* and *S. hurterii* in central Oklahoma. Since this difference in behavior appears to have a direct bearing upon ecological limitation of the geographic range of *S. hurterii,* it seems advisable to contrast the two briefly. Upon leaving the natal pool, a young individual of *S. bombifrons* seeks protection from desiccation by burrowing immediately into the mud near the water (see G. A. Moore, 1937). In very hot weather and bright sunshine this may occur even before the tail is much absorbed. For the next few days the young spadefoot remains in its burrow but may emerge at night, or after showers, to feed. It never allows itself to become dried out. Within a week or so the young animal wanders from the region of the pool, thereafter burrowing in any favorable spot each dawn after nights of feeding. From this time forward it exhibits essentially the habits of the adults.

In contrast, *S. hurterii* does not burrow immediately upon leaving the pool but seeks protection beneath leaves or other appropriate objects nearby. Even in very hot weather, the tiny animals refuse to enter the water again, once they are on the bank, and, if not shaded, will die by the thousands at the pool's edge, even on moist mud. Those that find protection beneath leaves rest there during the days immediately after metamorphosis but emerge at night to feed. In about one week, all suddenly disappear. I have not determined where they go. It seems likely that it is then that they begin burrowing but it may be only that they scatter widely after the manner of *Bufo a. americanus* in the same habitat. Thereafter they still emerge at night to feed and have been found rarely at this activity. From the first, however, they are more secretive than *S. bombifrons.*

The essential point is that *S. hurterii* could only under quite exceptional conditions emerge from a prairie pool such as constitutes the typical breeding site of *S. bombifrons,* because of a difference in the stage of development in the two species at which the burrowing instinct first becomes expressed. From this it follows that one of the limiting factors (perhaps the principal one) which keeps *S. hurterii* a woodland form is the inability of its young to metamorphose successfully in a prairie under the usual conditions obtaining there. Details in other species are not known sufficiently to say positively how they may fit into this picture, although Cope's (1889) observations suggest that *hammondii* is essentially like *bombifrons* in this respect.

(6) There is still one more phase in the breeding of the spadefoots which must be mentioned. In *Bufo*, the studies of Bragg (1940, 1940a), Blair (1941, 1942) and Bragg and Smith (1942) have shown that interspecific differences in breeding patterns, in breeding season and in calls play a large part in sexual isolation; and Blair particularly has emphasized these as isolating mechanisms among some of the eastern toads. In the prairie species (*i.e.,* those confined to prairies) the difference in breeding season is no factor at all, since all these follow the rains in breeding rather than the seasons (Bragg, 1940, 1940a, 1940b, Bragg and Smith, 1942, 1943). Call, differential behavior of the sexes and various differences in detail of breeding behavior, including differentials in selection of breeding sites, are very important in sexual isolation of various closely related and probably interfertile species.

The spadefoot toads are generally like the prairie-species of *Bufo* in these respects and have essentially the same types of isolating mechanisms. These mechanisms may all be conveniently grouped as either (a) physiological (or psychological), (b) ecological, or (c) geographic (so obvious as to need no further mention).

Physiological factors among the spadefoots involve principally a differential response to calls and a different pattern of male behavior in securing mates. The first of these has already been emphasized in other connections, but the second needs further elucidation.

The male of *Scaphiopus bombifrons* floats on the surface and calls repeatedly from one place without much moving about. Females are greatly attracted by this call, enter the pool and swim to the male, the latter paying little attention till touched by one of them, whereupon clasping occurs. The male of the closely related *S. hammondii* also calls from the surface; but in this case, the male swims about almost continuously while calling and rushes toward any other spadefoot in its vicinity attempting to clasp it (Ortenburger, 1925; Bragg, 1941c). If two males meet (and this is frequent) each struggles for position till one has attained it, but the clasped male is released immediately upon its croaking. Since females of *S. hammondii* also respond markedly to the call of the male, any female caught by a male is most likely to be of the same species. This difference in behavior appears to be the principal isolating mechanism between these two species when present together in the same pool. However, the possible segregation of species within adjacent pools under the influence of the voices of the first arrivals in each should not be overlooked.

Scaphiopus couchii and *S. hurterii* are similarly sexually isolated. The former most frequently calls on or near the banks of a pool, according to Ortenburger (1926), although it is not uncommon to find them calling while floating in the typical spadefoot manner. *S. hurterii* manifests the reverse tendencies, *i.e.*, whereas some may call from the bank (Smith and Leonard, 1934; Bragg, 1943) most matings appear to be at the surface of deeper water. As with *S. hammondii,* so with *S. hurterii* I have seen calling males rush at each other, struggle for the clasping position, and then separate immediately upon the croak of the unsuccessful member of the pair.

Since both sexes of *S. h. holbrookii* float at the surface and the male apparently awaits a female with little moving about (Ball, 1936), this species is probably sexually isolated from *S. hurterii* when breeding together (as is possible in Arkansas and eastern Texas) by the same factors which separate *S. bombifrons* and *S. hammondii.*

Ecological isolation is only partial in the spadefoots and may be looked upon as merely accessory to the psychological factors emphasized above. *S. bombifrons* commonly is found only in deeper pools. At Las Vegas, New Mexico, in 1940, I was struck by the fact that every pool in which *S. hammondii* alone was calling, as well as every pool from which I later secured tadpoles of this species only, was shallow; whereas, all deeper pools examined contained tadpoles of *S. bombifrons* and no *S. hammondii.* Pools of intermediate depth contained both species. Nevertheless, the most shallow pool of all contained only *S. bombifrons,* as explained elsewhere (Bragg, 1941c). I have also since found *S. bombifrons* in Oklahoma calling from shallow water. As mentioned earlier, *S. couchii* commonly breeds in much more shallow pools than *S. bombifrons. S. hurterii* uses both shallow and deep water apparently indiscriminately, but *S. h. holbrookii* seems from the accounts which I have read to be most common in deeper pools.

Tanner's (1939) account of *S. intermontanus* suggests that this species is also indiscriminate as to the depth of water. He says, "Great numbers in copula were observed at a small playa. . . ." This seems to indicate shallow water. On the other hand his statement, "Tadpoles were collected in the Price City Reservoir . . ." may imply fairly deep water. Concerning *S. multiplacatus,* we have only the statement of Taylor (1942) made in reference to tadpoles: "The shallow pool where they were collected was formed in an excavation where the earth had been removed in road building."

Another factor (in part psychological, in part ecological and in part physiological) tends to eliminate possible interspecific breeding. This is the already mentioned tendency for some species to be influenced more by heavy rains, others more by violent rains. This, however, is a minor factor, since it tends to be associated with a particular environment and, hence, to work potentially between only those species which occupy different habitats and are not likely to occur in the same pool. It may become important in areas where ranges overlap as in northern Texas, where *S. couchii's* range overlaps that of *S. hurterii,* or in east central Oklahoma, where the latter form meets *S. bombifrons* geographically (but not ecologically). In the latter situation, a few *S. bombifrons* inhabiting prairie islands may move into pools in adjacent woodland or savannah where *S. hurterii* breeds. After violent (but not particularly heavy) rains, *S. bombifrons* may breed here in small numbers without *S. hurterii* being affected. When, however, rains are both violent and heavy, the two species breed together. If, however, prairie islands have temporary pools within them then these are utilized by *S. bombifrons* and only very seldom by *S. hurterii.* In this case, the segregation and sexual isolation are entirely due to ecological factors.

Disregarding geographic isolation as so obviously an isolating mechanism as to need no special comment, we may summarize the above discussion as follows: even though all species of *Scaphiopus* follow a generally similar pattern of breeding, they are quite effectively sexually isolated even when breeding in the same pools. While several interacting factors play their parts, the two principal ones are (1) differences in details of how males secure females and (2) the attraction of the calls of the males for females of the same species.

PHYLOGENETIC INTERPRETATIONS

Those interested in speciation and other phases of organic evolution have quite naturally made most use of morphological variations in interpreting phylogenetic relationships and geographical distributions in groups with which they have happened to be dealing. Only rarely and quite recently have I noted much tendency to include habit-patterns in such interpretations except as quite incidental to the major morphological basis. Specifically within the anuran amphibians, taxonomists have based interpretations upon detailed measurements, color factors, other external features, and (more rarely) detailed differences in internal anatomy (muscles, skeletons, type and arrangement of teeth, etc.). Zoogeographers have also utilized such characters in correlating these with distribution as found in attempting to arrive at reasonable conclusions as to the origin and dispersal of this or that family or genus. Another group of scientists, smaller in number but of some significance, have looked into the habits of individual species or groups, studied the tadpoles, and elucidated other phases of life histories. One of the foremost of these is Wright (1914, 1929, 1931, etc.).

While each of these groups of students has added valuable contributions to our general knowledge within their respective fields and while all workers have realized that habits must play an important part in the lives of organisms in nature, very seldom has an attempt at a synthesis of all of these factors been made in tracing phylogenies. Habits particularly, partly through lack of knowledge, have often been neglected. For this reason habits are emphasized here.

The spadefoot toads are generally supposed to have arisen in southwestern North America and to have spread geographically from there, differentiating into the various species as they dispersed. Tanner (1939) says, "Geographically, the subgenera are fairly distinct, having evolved, it would seem, from a common center of origin near northern Mexico, the subgenus Scaphiopus having radiated into eastern United States, while the subgenus Spea is found in the western United States." Tanner offers no opinion as to the phylogenetic line followed within the subgenus Scaphiopus but, concerning Spea, he says, "The evolution of the subgenus Spea seems to be from *hammondii* through *bombifrons* to *intermontanus.*" This opinion was based principally upon a progressive change in the skeleton of the head but may have been influenced somewhat by the supposed closer resemblance of the mouth parts of the tadpoles of *inter-*

montanus to those of *hammondii* than to those of *bombifrons.* Actually, the *bombifrons* tadpole more closely resembles that of *intermontanus* than that of *hammondii* (see Bragg, 1941b) as also do those of *multiplicatus* as recently described by Taylor (1942). Of all the larvae within Spea, those of *hammondii* are the most distinctive. In some circles there is a tendency to emphasize embryonic and larval characters as more primitive than adult characters. On the basis of this alone, one would put *hammondii* at the *end* of its phylogenetic line rather than at its base. But one must not forget that larvae as well as adults must live in their native haunts and that this presupposes (at least as a possibility) specific *larval* adaptations to this end. In other words, it may be that the large jaw-muscles and peculiar mandible of the *hammondii* larva are specific larval adaptations and thus of no use in placing this species in its proper relationship to the others. This point awaits further study.

TABLE I

Some characteristics of the subgenus Spea as contrasted with the subgenus Scaphiopus. Italics are used to show wherein *S. couchii* departs from its taxonomic relatives and is more like members of Spea. It will be noted that, so far as knowledge goes, this is always at points where habits rather than morphology are involved. (?) = no conclusive date for *couchii:*

Spea (All in xeric habitats)	Scaphiopus (All, except *couchii,* in mesic habitats)
1. Eggs large.	1. Eggs small.
2. Eggs light in color.	2. Eggs dark in color.
3. Adults small.	3. Adults large.
4. Tadpoles large.	4. Tadpoles small.
5. No tendency of schooling of tadpoles at metamorphosis.	5. Tendency (under some conditions) to school at metamorphosis. (?)
6. Young burrow immediately on leaving pool.	6. Young delay burrowing upon leaving pool. (?)
7. Adults not secretive; common on roads at night.	7. *Adults very secretive seldom found even when searched for.*
8. Breed only after violent rains; amount of less significance than rate of fall.	8. *Breed only after heavy rains; rate of less significance than amount of fall.*

If now we turn again to the reproductive habits of members of the genus *Scaphiopus* and to specific ecological distributions, we find some very interesting phenomena (see also Table 1):

(1) All of Spea and one Scaphiopus (*S. couchii*) are found only in xeric habitats (grasslands of various types and deserts).
(2) All of Scaphiopus (except *couchii*) have more mesic habitats (woodlands, extensive flood plains, and the like).
(3) All species, regardless of their habitat now, show distinctly the xeric reproductive habit-pattern characteristic of desert and grassland anuran life.
(4) But all the genus inhabiting *mesic* environments differ from those which now occupy *xeric* habitats in characteristic ways (more secretive, initial breeding stimulated by heavy rather than violent rains, and perhaps in more delicate response to accumulation of soil moisture and to soil temperature).

These facts are best interpreted as follows (see also Fig. 6):

(1) The genus *Scaphiopus* arose in a xeric environment, presumably in southwestern North America as Tanner suggested.
(2) They lived there sufficiently long to have had the essentials of the xeric breeding-pattern become firmly fixed in their hereditary constitution probably through natural selection.
(3) The first bifurcation of the phylogenetic branch (into the subgenera Scaphiopus and Spea) occurred *before* they left this environmental situation.
(4) Later, all species of the Scaphiopus branch (except *couchii*) spread toward the east and north. In doing so, each changed its habits slightly so that a new sub-pattern tended to be developed in the more mesic situations.
(5) All species of Spea and one species of Scaphiopus (*i.e.*, *couchii*) remained in the original xeric environment and, having found the original habit-pattern good, so to speak, have not changed it materially in the thousands of years intervening.
(6) It follows that (a) the habit-pattern in breeding of any species of this group tends in evolution to adjust the species to some specific environment more or less independently of morphological changes usually interpreted as indicating phylogenetic diversity from other closely related forms; and (b) the suggestion that this general trend is manifested as well by other species (grass-land-limited species of *Bufo*, *Rana*, etc., as compared with woodland species) makes it reasonable to assume that the xeric-adapted forms of Anura in general have a convergence in habit-pattern more or less cutting across morphological diversities, and therefore, current lines of classification.

Such an interpretation as the above will explain why *S. h. holbrookii* of the East has always appeared so difficult to understand because it behaves so differently from all other associated frogs and toads. For here is an animal with the breeding habits essentially of a desert-inhabiting anuran, although at present limited to a mesic environment. It also helps to explain why *S. couchii*, although morphologically (both as adult and larva) belonging to the eastern group, is more like the species

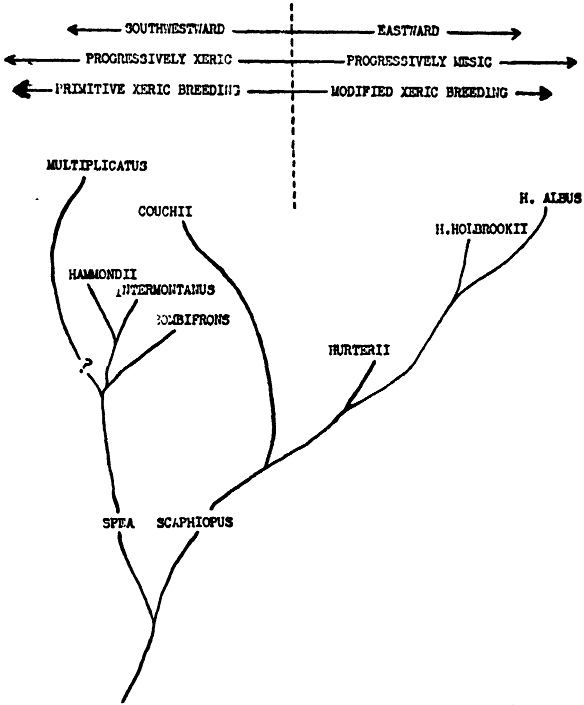

FIG. 6. Phylogenetic tree of Scaphiopus emphasizing habits and ecological relations. Of necessity, such a diagram must be somewhat oversimplified. For details, see text.

of Spea in habits than like its taxonomically closer relatives. This becomes all the more reasonable when we realize one more basic fact, namely, that a xerically adapted frog or toad can live without essential change in reproductive habits in a more mesic environment; whereas, in the reverse situation, the mesically adapted anuran could probably not survive long without change. This must necessarily be so for any animal depending upon standing water to rear its young, as is the case of most frogs and toads of North America. Any one who has observed at first hand the high plains or the desert country of the North American Southwest under the influence of a summer sun will have no difficulty in appreciating this fact and this is equally true of those who have seen the normal pattern of rainfall in this region, veritable torrents of rain when it does come, filling every hollow with suitable, sometimes quite extensive but always quite temporary, breeding sites. Hurried development of larvae, so characteristically a part of the xeric breeding-pattern as exhibited by the spadefoot toads, is an absolute necessity to the survival in such a rigorous environment whereas this same feature is not a detriment to development in a more mesic situation; hence so little change in Scaphiopus of the East as compared to *S. couchii* and Spea of the Southwest. Carnivorous and cannibalistic tendencies in the tadpoles is another case in point, for the temporary pools formed by the torrential rains of the high plains and deserts are fairly barren when compared with pools of similar extent and volume in more mesic regions, such as the East. This is so, despite assertions of Gilmore (1924) and myself (Bragg, 1940) as to the varied life in such pools because the greater part of this life is of animal nature and all types are in very fierce competition for the few plants which, as always, are the ultimate food source. Partly carnivorous feeding habits are a distinct advantage to any tadpole under such circumstances and this also explains why members of the crustacean genus, *Apus,* a common inhabitant of the temporary pools of the short-grass country, has been seen feeding upon tadpoles of *S. bombifrons,* although it belongs taxonomically to a group usually feeding upon algae. Again, these habits are not particularly detrimental to frogs and toads in mesic situations and so have not been eliminated by natural selection in *S. h. holbrookii* of the East. In fact, as a generalization, it is clear that almost any specialization in habit-detail which adjusts a frog or toad to a xeric habitat will not be particularly detrimental to it in a moister climate and therefore will not likely be changed by natural selection if the species spreads to, or is introduced into, a more mesic situation. It is equally true that any habit-detail specifically adapting a frog or toad to a mesic habitat tends to serve as a barrier to its spread into a more xeric situation, unless it is able to do so with change toward the xeric pattern. It follows that, the climates of North America being what they are, it is easier for a western species of Anura to move eastward than for an eastern species to extend its range westward on this continent unless advantage be taken of special conditions and special restrictions to quite local ecological situations or unless habits are changed at the right places to be effective for survival.

SUMMARY

Through seven years, the peculiar advantages of the climate, ecological associations and geographic location of Oklahoma have been used in a study of the habits and distribution of Scaphiopodid toads. Four of the eight known forms occur and every opportunity for their study in all parts of the state has been utilized. Most of this has been in the field at night. Thousands of miles have been traveled and eggs and tadpoles have been studied in the laboratory. This work is here summarized and correlated with that of others in such a way as to form a summary of our knowledge of the group at the present time. The principal conclusions are as follows:

(1) Tanner's (1939) conception of the two species groups as representing two subgenera, Spea of the west and Scaphiopus of the East, is accepted and confirmed by details concerning tadpoles and habits.

(2) Tanner's further conclusion that the Scaphiopodidae arose in the North American Southwest is confirmed, also principally by the study of habits.

(3) It is clear that Anuran amphibians in North America which are limited to grasslands and deserts in their distribution have a breeding pattern different from those not so limited. Two principal patterns are described, the xeric and the mesic.

(4) The xeric pattern includes the following tendencies: (a) the lack of a definite breeding season; (b) use of temporary water only; (c)

initiation of breeding behavior in nature only by the coming of rain; (d) a hastened development of larvae; (e) possession of rather loud voice by the male; (f) marked attraction of males as well as of females by the breeding call of the species, thus tending to build up large congresses very quickly.

(5) The mesic pattern includes the opposite tendencies in various degrees and is the common one of eastern species in contrast to those limited to the western grasslands and deserts.

(6) All spadefoots show the xeric pattern exclusively.

(7) However, those in mesic environments vary slightly, but consistently, from those still in xeric environments (*i.e.*, they have developed a subpattern still essentially xeric in nature).

(8) Since a xeric pattern in a mesic environment would not likely be detrimental to the life of a frog or toad, whereas a mesic pattern in a xeric environment in all probability would be, it follows that (other things being equal) eastern forms can spread westward only slowly and with difficulty whereas western species can spread eastward more easily. This, of course, could be modified by other conditions.

(9) Applying these things to the spadefoots, we infer that they arose in a xeric environment, became adjusted nicely to this through natural selection, and split into two subgenera. Then one of these (Scaphiopus) tended to spread eastward and northward (*i.e.*, to mesic conditions), changing in reproduction habits only slightly; whereas the other (Spea) remained in xeric regions without essential change in habits.

(10) The one exception (*S. couchii*) is one that proves the rule; for, although belonging to the subgenus Scaphiopus taxonomically it nevertheless remained in a xeric habitat with members of Spea and retained the original habit-pattern like these.

(11) From this it follows that (so far as North American anuran amphibians are concerned) the habit-pattern in breeding does not necessarily follow taxonomic lines during speciation and dispersal to varied environments. While this is shown most clearly by the spadefoot toads, there is ample evidence of it in other genera as well.

(12) The emphasis in habit-patterns above deals only with similarities; but interspecific differences should not be overlooked. Details given in the text show clearly that each species is different in some respects from all others (even in habits). The situation seems to be that (a) all species differ in details of habits, (b) each tends to be adjusted to reproduce in some one environment or several closely related environments, (c) those inhabiting the same ecological (not necessarily geographic) situation tend (regardless of taxonomy) to develop the same or essentially similar habit-patterns (convergent evolution) and (d) those even closely related taxonomically (and with widely overlapping geographic distribution) which are adjusted to essentially different ecological situations, tend to be different in habits and habit-patterns, even in the same geographic locality.

(13) Spadefoot toads which inhabit the same region (ecologically as well as geographically) tend to be sexually isolated during breeding by two

types of factors, (a) psychological and (b) ecological. Psychological isolation is the more important and is made effective through two phenomena, the differences in behavior of the males in securing mates and the marked interspecific attraction of spadefoot breeding calls. Ecological isolation is only partial and involves differential responses to amounts of rain as well as possible specific selection of breeding sites. The latter may be more psychological than ecological, however, due to the marked attraction for others of the call of the first spadefoot to arrive at a given pool.

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REVIEWS AND COMMENTS

EDITED BY PROFESSOR CARL L. HUBBS

In these reviews and notices of current biological publications emphasis is given to books and major articles which fall within the special scope of THE AMERICAN NATURALIST, in that they deal with the factors of organic evolution. REVIEWS AND COMMENTS are meant to include also such general discussions, reports, news items and announcements as may be of wide interest to students of evolution. Except as otherwise indicated, all items are prepared by the Section Editor, Dr. Carl L. Hubbs, Scripps Institution of Oceanography, La Jolla, California. All opinions are those of the reviewer.

Evolution, Creation, and Science. By FRANK LEWIS MARSH. Washington: Review and Herald Publ. Assoc., 1944: 1–304. $2.25.

THIS book accomplishes what this reviewer believed, before having read it, to be impossible. It would seem that in our day an anti-evolutionist must of necessity be either ignorant of the evidence or incapable of sound reasoning. Yet, this book gives a tolerably readable review of the pertinent facts, and then, by means of what on the surface appears to be sensible argumentation, reaches the conclusion that this evidence favors the theory of special creation and not of evolution. According to "American Men of Science," the author holds a Ph.D. degree from the University of Nebraska; he is also the author of several original investigations published in reputable biological periodicals. But he is convinced that the world and man were created in six days of usual length. The way this legerdemain is accomplished is in itself interesting as an example of high variability of human thinking processes.

The author must be commended for making his premises explicit. To a special creationist, the "approach to biological research must be with impartiality in every point except with regard to those basic principles laid down in the Bible." Among the "basic principles" he finds that the world began its existence about 6000 years ago; that sometime thereafter all land animals, except those which found refuge in the Ark, were destroyed by a flood; that the now living land animals are all descended from ancestors dispersing from Mount Ararat in Armenia as the center. What a pity that some otherwise efficient intellects are endowed with imagination too shallow to see that great truths can be expressed in poetry and myths!

However, the author rejects the view that all existing species and races were created and placed where they are found to-day. On the contrary, he regards it as established by scientific evidence that great changes and diversification occurred since the flood. What was created were not races or species (the word "species" appears always in quotation marks) but "kinds" (without quotation marks). The definition of "kind" is evidently a crucial matter. We are told that any two forms which are capable of producing a hybrid zygote, even though the hybrid may die in the earliest stages of development, belong to the same "kind," but that failure to cross does not always signify difference in "kind." A "kind" may include not ony several races and species but even genera and families. But in one of the concluding chapters we read "I hold that it is quite impossible to-day to tell which forms are members of an original kind." The reviewer feels that the last sentence contains an implicit blasphemy, for it imputes meanness to a creator who would play such a trick on unsuspecting humanity. Yet, this unsolvable puzzle gives comfort to the author, because it permits him to accept all the evidence experimental biology has to offer for evolution—to him any change is always mere modification within a "kind." In fact, he outbids evolutionists on the score of the speed of the changes, for he assumes that all dogs, foxes, and hyenas are members of a single "kind," and, therefore, must have descended from a common ancestor in any event less than 6000 years ago. The possible mechanisms of such changes are discreetly left in the dark; the author obviously does not like natural selection, which is not even given a few lines of explanation.

The acceptance of the mysterious "kinds" does not, however, take care of the evidence for evolution derived from comparative morphology, embryology, paleontology and geographic distribution. These problems are dealt with each in a separate chapter, the reading of which perhaps leads one to admire the author's intellectual bravery but does not add much to the attainments of his predecessors in this field. It is, incidentally, surprising that the author ignores the writings of distinguished biologists like Wasmann who have also argued for evolution within but not beyond the limits of variously conceived "kinds." The chapter on paleontology is, as one may expect, particularly startling. Among other interesting things, the reviewer has learned from it about the existence of the periodical named "The Bulletin of Deluge Geology and Related Sciences" which is being published in the United States in the twentieth century (an issue of 1942 is referred to in the book).

A valuable lesson can be derived from reading the book under review. The attractiveness of science to many people lies in that it seems to answer the indestructible yearning for certainty so complete that no thinking mind can evade its acceptance. We like to believe that if we secure adequate data bearing on a scientific problem, then anybody with normal intelligence who takes the trouble to become acquainted with these data will necessarily arrive at the same conclusion regarding the problem in question. We like to speak of conclusions demonstrated, settled, proved and established. It appears, however, that no evidence is powerful enough to force acceptance of a conclusion that is emotionally distasteful.

TH. DOBZHANSKY

Contributions to the Genetics, Taxonomy and Ecology of Drosophila pseudoobscura and Its Relatives. By TH. DOBZHANSKY AND CARL EPLING. Carnegie Institution of Washington Publication 554, 1944: i–vi, 1–183, pls. 1–4, 24 figs. $2.25 (paper), $2.75 (cloth).

THROUGH a series of recent researches, among which those of zoologist Dobzhansky and botanist Epling are outstanding, *Drosophila* has been elevated to a position of pre-eminence in speciation.as well as in genetics. Such work is effectively filling in the chasms that long split biology into zoology and botany,. and each into systematics and genetics, into morphology and physiology, into "closet biology" and "natural history," into observational and experimental subsciences. The way is now being cleared for more unified advances, with more penetrating and more secure attacks on such central problems as evolution.

A skillful combination of genetic experiment and of cytological observations on the giant salivary chromosomes has disclosed races of *Drosophila* that are characterized by different arrangements of the gene-file along one or more chromosomes. It is shown by a brilliant analysis (1) that the several arrangements can be attributed plausibly only to series of inversions and (2) that their phyletic sequences can be postulated with little doubt. This is something distinctly new in a phylogeny that is unsupported by a fossil record. The chromosomal type has been determined for an immense number of individuals representing many populations over western North America and in Mexico and Guatemala. As a consequence it has been possible to analyze the data along several west-east and north-south transects. These exhibit very definite clines, and permit reasonable inferences in regard to the origin and spread of the genetic types.

In two respects such chromosome races appear to have only a limited bearing on the speciation process as it is generally interpreted. In the first place the chromosome types do not seem to involve other differences, such as might be employed in routine systematics. Secondly, since the chromosomal arrangements are indicated (by a clever statistical analysis) to have little or no selectional significance they are probably not involved in adaptive speciation, which presumably is the usual sort. Nevertheless the thorough analysis of these chromosome types proves very significant.

The chromosome races illustrate beautifully the genetic heterogeneity of species. They are so numerous as to be practically uncountable, occupy very restricted areas and are constantly changing. These and other races are interpreted as genetically open systems through which genes of other conspecific races are free to pass. They are defined in Dobzhanskian style as "populations characterized by different frequencies of the variable genes and chromosome structures."

There appears, however, to be some inconsistency in Dobzhansky's concept of races. After stating that different genes or gene arrangements may flow independently through a species, he asserts that "an individual may, in this sense, belong to two or more races at the same time." But if, as Dobzhansky writes, races are groups of individuals, not single individuals nor single genotypes, then each individual can belong to only one population, except in so far as that population may be split into smaller aggregates or lumped into larger ones. The evolutionary organization of races, by which the characters of each are rendered increasingly uniform and distinctive, appears to be somewhat slighted in studies, like the present one, which are focused on the genetic mechanisms.

With this background of race analysis (written by Dobzhansky) and of the studies on taxonomy, distribution, ecology, dispersal, seasonal cycles and general natural history (worked out by the two authors in collaboration), Epling speculates boldly and interestingly on the ancient history—we might say prehistory—of raciation in *Drosophila pseudoobscura* and its allies. Leaving an area of solid ground where every concept rests on an abundance of fact, he steps out onto the bog of hypothetical phylogeny. He does not, however, sink wholly out of sight, as many have done on the same ground. Before postulating an historical basis for the present organized pattern of race distribution he plausibly disposes of alternative explanations: wide dispersal by winds, separate origins of each race, and natural selection of chromosome types. Reviewing data on the geological history of the west he abandons the opinion of Dobzhansky and Sturtevant (1938) that the present distribution of *Drosophila* races was determined in relatively recent, perhaps Pleistocene or post-Pleistocene time. Regarding the Glacial climate of the west as having been too much like that of the present to have permitted the establishment of the observed distributional patterns, he dates the origin of races differing only in chromosome arrangement from Miocene or earlier. This seems to me to be an inherently inprobable estimate, and my studies of fish distribution in the Great Basin and elsewhere lead to the view that during late Glacial time the whole western region was sufficiently cold (not warm) and moist to allow the establishment then of the present distribution of the *Drosophila* races.

Speed in Animals. Their Specialization for Running and Leaping. By A. BRAZIER HOWELL. Chicago: University of Chicago Press, 1944: i–xii, 1–270, figs. 1–55. $4.00.

"SPEED in Animals" is a catch title, obviously designed to help sales rather than to indicate precisely the material treated. The anatomical basis of locomotion in mammals is the real subject. Nothing is included on the speed or on other phases of the locomotion in the invertebrates, which constitute the vast majority of animal species. There is no statement on the maximum speed attained by fishes in swimming, though data are available. The optimum stream-lined form of certain fishes and of other fast-moving animals is inadequately emphasized. In fact fishes and amphibians are treated primarily as the donors of a structural heritage which limited the locomotor adaptations of the higher vertebrates. Reptiles and birds are likewise accorded only a few pages.

As one might expect from his leadership in the field of the comparative anatomy of the group, the author discusses the locomotion of mammals at much greater length. His prime contribution is a synthesis of mammalian anatomy and behavior. He clarifies the steps by which skeletal and muscular structures were modified in keeping with particular locomotor needs. He thus makes a notable contribution to functional anatomy.

Under "Proportions" there are offered several percentage indices which quantify the relative lengths of parts that are differentially elongated in swift mammals. No indication is given, however, of the variability of the indices within a species.

A particularly detailed treatment is accorded the gaits of mammals, particularly of horses. This section should prove of interest to horsemen.

In general the anatomy and phylogeny of the vertebrates seem to be presented soundly, though only one of two or more conflicting theories is sometimes presented. Occasionally one encounters an incomprehensible suggestion, such as the idea that the abdominal and thoracic pelvic fins of different teleost groups are nonhomologous.

In regard to such controversial subjects as evolutionary processes Howell states that he has followed his own beliefs in preference to presenting all sides of the argument. The seemingly ineradicable ghost of Lamarck repeatedly wafts its way over the pages of the book, as on page 1, where the stimulus for the original formation of the paired fins from scales (a questionable phylogeny) is stated to have probably come from "wriggling over the bottom and between stones in search for food."

NOTICES OF NEW ARTICLES AND BOOKS

Ends as Well as Means in Life and Evolution. By EDWIN G. CONKLIN. Trans. N. Y. Acad. Sci., (2) 6, 1944: 125–136.—"I have no theory to propose to explain the origin of organic fitness and human purpose other than the endowment of living beings with differential sensitivity and reactivity, tropisms, organic memory, trial and error behavior, leading in higher animals to intelligence and purpose. . . . But with crude mechanism that finds everything the result of accident and chance I have no more sympathy than with transcendental vitalism. Those who say there are no ends, values, purposes in the living world may understand atoms, molecules and even genes, but they do not understand organisms."

Geographical Variation in *Rana pipiens* Schreber of Eastern North America. By JOHN ALEXANDER MOORE. Bull. Am. Mus. Nat. Hist., 82, 1944: 345–370, pls. 61–66, figs. 1–3. $0.60.—With an uncommon degree of thoroughness and precision Dr. Moore has tackled one of the most perplexing problems in the systematics of American amphibians. He demonstrates clearly the inadequacies of prior attempts to separate the *Rana pipiens* complex into species. His work, in fact, is more a critique of previous systematic interpretations than a fresh attempt to define the subspecies—probably many, rather than the two or three customarily considered—into which *pipiens* may prove divisible. His own data, as well as unpublished studies of others, strongly suggest that such local forms can be recognized. A particularly interesting new character brought out in this paper is the usual presence in certain regions of an oviduct in adult males. Chiefly by this feature one might separate a subspecies inhabiting the distinctive faunal area of peninsular Florida. This form is shown to be rather strongly isolated sexually. Other regional differences in biology have been demonstrated by Moore's thorough studies. The existence is suggested of ecotypes on a scale too small and in a pattern too complex to warrant their recognition as subspecies. Since such peculiarities as well as great individual differences seem to dominate the variational picture, Moore makes the thought-provoking suggestion that it would be unwise to delimit subspecies on the basis of the fewer and smaller differences that happen to be correlated with geographical distribution. There is logic in this unconventional idea, the application of which, however, would spread havoc in systematics.

A Preliminary Study of the Thermal Requirements of Desert Reptiles. By RAYMOND BRIDGMAN COWLES AND CHARLES MITCHILL BOGERT. Bull. Am. Mus. Nat. Hist., 83 (5), 1944: 261–296, pls. 19–29, figs. 1–3. $0.70.—Among the conclusions reached in this careful study we may note: The maximum voluntary temperature tolerance of the desert reptiles studied is 42° C., not far below the lethal threshold. Nocturnal reptiles tolerate and prefer lower temperatures than do the diurnal species. Extensive fluctuations are avoided. "Thermoregulation by means of behavior may be considered an element of basic importance in the existence and evolution of reptiles in continental climates." Survival of reptilian relicts on islands and peninsulas is perhaps attributable to the slight temperature fluctuations in maritime climates. "An evolutionary compromise may exist when antagonistic forces are involved."

The Wolves of Mount McKinley. By ADOLPH MURIE. Fauna of the National Parks of the United States. Fauna Series, No. 5, 1944: i–xix, 1–238, frontisp., figs. 1–58.—The interrelations of the wolf and the handsome Dall sheep of Mt. McKinley National Park were thoroughly studied from 1939 to 1941 by one of our most capable wildlife researchers. He concludes that the wolves are the main factor in stabilizing the numbers of the mountain sheep; that is, in preventing an overpopulation. The caribou, however, is the main food of the wolf, and serves as a buffer species for the Dall sheep. Evidence is presented to indicate that it is the weak, old and young sheep that are preyed upon. The slowest and weakest caribou calves are the ones that are caught by the wolves. Selection for speed and strength in the young appears to be intense.

"It must be emphasized that national parks are . . . areas dedicated to preserving samples of primitive America," we are told, and Adolph Murie describes such a sample most interestingly. His brother Olaus, an accomplished artist as well as naturalist, has further enlivened the text with his action drawings.

Ancient Man in North America. By H. M. WORMINGTON. The Colorado Museum of Natural History [Denver], Popular

Series, No. 4, Second (Revised) Edition, 1944: 1–89, 14 figs. $0.35.—Though written for the comprehension of the interested, intelligent layman, this treatise is so thorough and so judicious as to be of high value to scientists. The conclusion is reached, and seems inevitable on the basis of the scattered evidence here amassed, that man has inhabited North America for at least several thousand years—a much longer period than many anthropologists only a few years ago would have allowed. Indications that the continent was inhabited far earlier than early Postglacial or late Glacial times, however, is discounted. It is particularly clear that sections of the now arid West supported a human population during times when the supply of surface water was much greater and more constant than it now is. At least some of these times were the pluvial periods that are to be synchronized with montane glaciation and very probably with continental glaciation. There can no longer be any reasonable doubt that early Americans were contemporaneous with now extinct mammals long regarded as distinctively Pleistocene. Just when these animals became extinct, however, is admittedly an unsolved problem.

The Naturalist's Lexicon. By Robert S. Woods. Pasadena: Abbey Garden Press, 1944: i–xviii, 1–282. $2.75 (7 cents postage.)—With the decline, often the elimination of classical training, scientific names have become to most biologists a mere jumble of letters hard to learn and pronounce. This is particularly true of zoologists, many of whom, even some systematists, accept with equanimity or actually take pride in crude abuses of two inherently beautiful languages. They overuse barbarisms, allow bastard combinations of Greek and Latin and as a rule concoct unnecessarily long, awkward or meaningless names. They miss the pure joy that comes from understanding and especially from coining a name that is short, euphonious and meaningful. Recognizing this state of affairs Mr. Woods has painstakingly prepared for naturalists a manual for scientific names. He starts with a brief but useful discussion of the principles of word formation as applied to systematic nomenclature, then devotes the major portion of the book to a lexicon combining Greek words, arranged according to the Latin transliteration, and Latin words; in all a selection of about 15,000 classical words used or usable in systematics. He closes with a "Condensed and Classified English-Classical Supplement," arranged primarily as to parts of speech. This last section is potentially the most useful to working systematists, but in the first edition I think is too far condensed to serve the maximum good. I have often felt the need and once started to prepare a more extensive and more thoroughly classified name-finder for those who describe new genera and species. I would have given the combining forms without any separation of the parts of speech. Mr. Woods's book will go far, but a second edition with an expanded classified list would go farther toward the enrichment and refinement of biological nomenclature.

The Life Energy of Species. By HAROLD ALEXANDER DANNE. New York: privately printed by author, 131 West 98th St., New York, N. Y., 1944: 1–28, 4 figs. and 1 folded chart (issued in editions bearing on the wrapper such titles as Oil Identification and Wool Identification, differing only in the folded chart). No price stated.—If verified the views of engineer Danne would constitute a revolution in evolution and systematics as well as engineering. By the use of the "Time-energy Cell" and appurtenant apparatus, procurable from the author with directions that are not elaborated in the booklet, it is claimed that one can obtain evidence on "the dynamic origin of species and their composition, growth and propagation." Since water is the carrier of the life energies, the determination of "Hydrol or Moisture" seems to be an important step in the analysis of species.—The whole is quite beyond the comprehension of the reviewer.

A Life of Travels. By C. S. RAFINESQUE. Being a verbatim and literatim reprint of the original and only edition (Philadelphia, 1836). Foreword by Elmer D. Merrill. Critical Index by Francis W. Pennell. Chronica Botanica, 8, 1944: 291–360, pls. 5–8, 1 fig. Waltham, Mass., The Chronica Botanica Co.; New York, G. E. Stechert and Co. $2.50.—The brilliant, erratic naturalist Rafinesque will live more clearly in the minds of those who may now, thanks to the editors and publishers, read his autobiography. Through this medium he will invoke in modern naturalists a mixture of understanding, sympathy, pity and humor, as he is followed through his early travels in Europe and his extensive journeys, largely on foot, through the northeastern quarter of the United States. Again and again he proves what he rather tragically realized, that he was far ahead of his time. Though his reflections were as hasty and erroneous as were his observations, some of his concepts were prophetically brilliant. Thus we learn in the Foreword that as early as 1832 "he forecast the general principles of organic evolution in his published statement: 'The truth is that Species, and perhaps Genera also, are forming in organized beings by gradual deviations of shapes, forms and organs, taking place in the lapse of time. There is a tendency to deviations and mutations through plants and animals by gradual steps at remote irregular periods. This is a part of the great universal law of perpetual mutability in every thing.' "

Thomas Jefferson and the Scientific Trends of His Time. By CHARLES A. BROWNE. Chronica Botanica, 8, 1944: 361–424, illustr. Waltham, Mass., Chronica Botanica Co.; New York, G. E. Stechert and Co. $1.25.—Jefferson is pictured as one whose inclinations were more toward science than toward politics, into which he was drawn by the events of his time. His scientific interests were versatile and his effect was great on the development of science in America. His approach to scientific problems was predominantly utilitarian and in his judgment of new scientific ideas he was highly conservative. In fact at times the great champion of human liberty might well have been called a scientific tory.

SHORTER ARTICLES AND DISCUSSION

INTERACTION OF ALLELES AT THE BOBBED LOCUS IN DROSOPHILA HYDEI

An analysis of samples taken from *Drosophila hydei* populations has shown that a complex series of alleles occurs at the bobbed locus (Spencer, 1938; in press). During the course of this analysis most of the wild flies tested were mated only to one bobbed tester stock. Such experiments were adequate to demonstrate the genetic variability existing at the bobbed locus, but were not designed to furnish critical evidence on the gene-substrate theory recently developed by Stern (1943; Stern and Schaeffer, 1943; see also Wright, 1941). However, in the course of the bobbed investigation one small experiment was set up, the results of which are of interest in this connection.

Twenty-two males, all phenotypically normal, collected from a citrus dump near Azusa, Southern California, were each mated separately to females from a Wooster bobbed tester stock, the females of which had bristles of grade 20 (posterior scutellars about 400 microns in length); these same males were then mated to females from Berkeley bobbed tester stock, the females of which had bristles of grade 28 (posterior scutellars about 590 microns in length). The maximum bristle grade of females of this species is 33 (posterior scutellars about 690 microns in length).

In Table 1 the results of these tests are given. It will be noted that 7 of the males tested gave phenotypically bobbed daughters with the Wooster grade 20 tester and one of the males gave pheno-

TABLE 1

PHENOTYPIC BOBBED GRADES OF THE HETEROZYGOUS DAUGHTERS OF 22 WILD AZUSA MALES MATED FIRST TO WOOSTER BOBBED 20 FEMALES AND THEN TO BERKELEY BOBBED 28 FEMALES

Male No.	Bristle grades of daughters from Wooster 20 matings	Bristle grades of daughters from Berkeley 28 matings
1	30	33
3	30	33
14	31	33
19	31	27
20	26	no offspring
21	27	33
22	25	33

Note: All other of the 22 males tested produced daughters with maximum bristle grade 33 in crosses to both testers except No. 10 which gave no offspring in the Berkeley test. Grade 25 has posterior scutellars about 525 microns long and the maximum or normal grade 33 has posterior scutellars about 690 microns long.

typically bobbed daughters with the Berkeley grade 28 tester. The tests of the one male, No. 19, which gave phenotypically bobbed daughters with both Wooster and Berkeley testers do not fall into line with any simple interpretation in terms of heterozygous combinations with intermediate effects. The heterozygote of this wild Azusa gene with the presumably weak bobbed, *i.e.*, nearly normal, Berkeley grade 28 gene is a more extreme bobbed of grade 27 than the heterozygote of grade 31 formed by the Azusa gene and the Wooster grade 20 gene.

This case might well be explained by the gene-substrate interaction theory. In its simplest form this theory postulates two gene properties, one a combining power of the gene with a cellular substrate, and the other an efficiency factor in terms of the utilization of the substrate in the production of a substance for the development of the normal phenotype.

The facts concerning bristle phenotype in this case may be summarized thus, using the symbols b^W, b^B and b^A for the Wooster bobbed 20 gene, the Berkeley bobbed 28 gene and the Azusa wild gene respectively:

$b^W b^W$ = grade 20 female.
$b^B b^B$ = grade 28 female.
$b^W b^A$ = grade 31 female.
$b^B b^A$ = grade 27 female.

An interpretation of these facts in terms of gene-substrate interaction offers several possibilities. In the absence of additional information these can not be discriminated from one another. They all agree in attributing relative variations between the alleles in respect to both combining power and efficiency. One of these possible interpretations might be summarized thus:

b^W—low combining power; low efficiency.
b^B—high combining power; low efficiency.
b^A—low combining power; high efficiency.

On these assumptions the heterozygous combination of b^A with b^W would be expected to produce longer bristles than the heterozygous combination of b^A with b^B even though homozygous b^W has shorter bristles than homozygous b^B. The other Azusa genes which gave phenotypically bobbed heterozygotes with Wooster bobbed 20 but not with Berkeley bobbed 28 might be thought of as having high combining power and high efficiency. The several heterozygous combinations would then be determined by gradations in the two gene properties interacting to produce the phenotypes observed. It is not likely that either the combining power or efficiency of a series of alleles can be fully described in terms of only two levels, a high and a low one, but rather by a series of levels including several to many intermediate states between the highest and lowest. A case apparently similar to the one described here has been reported by Goldweber (1939) for allelic interaction at the white locus in *Drosophila melanogaster.*

TABLE 2

MULTIPLE PHENOTYPIC EFFECTS IN THE HETEROZYGOUS DAUGHTERS OF 11 BOBBED FEMALES COLLECTED FROM AZUSA CITRUS DUMP AND MATED TO MALES FROM WOOSTER BOBBED 20 TESTER STOCK

Female No.	Offspring Sex Bristle grade (n) normal abd. (ab) abnormal	Emergence records Counts at 1–2 day intervals				Viability-Total counts		
						Males	Females Higher grade	Females Lower grade
h109	♂♂ 31 (n)	9	18	33	35	95		
	♀♀ 30 (n)	5	5	22	7		39	
	♀♀ 14 (n)			8	18			26
h111	♂♂ 32 (n)	10	12	32	14	68		
	♀♀ 27 (n)	1	12	8	5		26	
	♀♀ 19 (n)		2	12	12			26
h113	♂♂ 33 (n)	12	27	11		50		
	♀♀ 26 (n)	2	7	4			13	
	♀♀ 13 (ab)		2	6				8
h114	♂♂ 30 (n)	17	16	25	26	84		
	♀♀ 28 (n)	8	6	28	10		52	
	♀♀ 17 (n)	4	0	14	14			32
h116	♂♂ 30 (n)	2	22	37	23	84		
	♀♀ 22 (n)	1	0	28	19		(24) 48	(24)
h117	♂♂ 31 (n)	8	17	7		32		
	♀♀ 18 (n)	13	30	4			(23) 47	(24)
h118	♂♂ 31 (n)	19	7	47	24	97		
	♀♀ 23 (n)	5	8	22	11		46	
	♀♀ 12 (ab)			4	9			13
h120	♂♂ 30 (n)	12	4	31	26	73		
	♀♀ 27 (n)	2	2	18	6		28	
	♀♀ 20 (n)	1	3	20	13			37
h131	♂♂ 31 (n)	7	15	36	23	81		
	♀♀ 26 (n)	2	7	19	7		35	
	♀♀ 19 (n)		7	19	15			41
h132	♂♂ 32 (n)	1	1	26	22	50		
	♀♀ 26 (n)	2	8	12	7		29	
	♀♀ 21 (n)			15	5			20
h140	♂♂ 30 (n)	7	16	29	16	68		
	♀♀ 28 (n)	1	10	10	9		30	
	♀♀ 22 (n)	1	5	19	5			30
					Totals . .	♂♂ 782	♀♀ 345	♀♀ 281

Further evidence that the action of alleles at the bobbed locus in *D. hydei* can not be fully described in terms of a simple potency series with heterozygous combinations showing intermediate phenotypes is derived from a study of pleiotropism at this locus. In Table 2 data are presented on bristle length, condition of abdominal sclerites, emergence time or time of larval-pupal development, and larval-pupal viability among the daughters of 11 phenotypically bobbed females of different grades collected from the Azusa population and mated each to a Wooster bobbed 20 tester male.

Each of the 11 cultures was reared in a large glass vial, using the Kleenex paper-yeast suspension technique described by the author (Spencer, 1943). This culture method ensures a good food supply and uniform culture conditions. However, in some cultures a certain amount of crowding occurred. The best index of this crowding is the size of the sons as determined by the correlated bristle length, which is in the male independent of the bobbed gene carried in the X-chromosome.

All the females listed except h116 and h117 carried two alleles forming phenotypically distinct heterozygotes in combination with Wooster bobbed 20. In general it will be noted that the more extreme of the two bobbed bristle types among the heterozygous daughters in a culture are less viable and have a longer larval-pupal period of development than their sisters. However, the daughters of h117 of the rather extreme grade 18 emerge as soon as do their brothers and appear to be somewhat more viable. While most of the heterozygous combinations listed in the table show normal abdominal sclerites even when of bristle grades as low as 14 the Berkeley bobbed tester of grade 28 referred to above shows abnormal abdomen. It is obvious from these and other observations made on the *D. hydei* bobbed complex that the seriation of various allelic combinations for one phenotypic character is not paralleled by that for certain others. These facts suggest for their explanation assumptions similar to those made by Stern and Schaeffer (1943) in interpreting the multiple effects of certain alleles at the cubitus interruptus locus in *D. melanogaster*.

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THE EFFECT OF REMATINGS ON THE FECUNDITY OF AN INFERTILE MUTANT FEMALE

FEMALES homozygous for *glossy*, an allele of *lozenge* in *Drosophila melanogaster*, are usually sterile. Glossy is a sex-linked recessive trait which is expressed by modification of eye-pigment, abnormal structure of the eye and an absence of the spermathecae and parovaria. Oliver and Green (1944) reported that only 23 per cent. of 899 viable glossy females produced offspring and that those which were fertile produced on the average approximately 9 offspring. The fertile glossy females produced more eggs than offspring. Although they produced offspring only during the first three days following matings, they continued to deposit eggs after that time. Even on the days the females were fecund, many of the eggs failed to hatch. It is apparent, therefore, that for some reason the eggs deposited 72 hours or more after the female has been inseminated do not become fertilized. In so far as can be told by microscopic examinations, the eggs of glossy females do not differ from those of normal females. Glossy females lack two structures of the internal genitalia, the paired spermathecae and parovaria. No visible difference between the ventral receptacle of normal and glossy females can be observed. The suggestion was made by Oliver and Green that the loss of fecundity by glossy females is probably due to a physiological change in the female which causes the stored sperm to lose their ability to fertilize eggs.

Although the eggs produced by glossy females can not upon examination be distinguished from normal eggs, the loss of fecundity by the glossy females might be due to changes in the eggs effected by increased age of the females. Matings have been made to test that possibility. Glossy females were mated, allowed to lose their fecundity, and then mated to another group of males.

Assistance to the study has been given by a grant in aid from the University of Minnesota Graduate School.

METHODS

Virgin homozygous glossy and wild-type females, not over 48 hours old, were mated individually to wild-type males in vials containing cornmeal-agar culture media. One group of both types of the females was tested to determine how long the females remained fertile after a single mating. After the females had mated, the males were killed. At intervals of 24 hours through a nine-day period, the females were transferred to vials of fresh media. Counts were made of the offspring produced for each 24-hour period.

Another group of the females was mated first to wild-type males and, subsequently, to Hairless-Moire (*H/Me*) males. Hairless and Moire are dominant traits that are associated with the third pair of chromosomes. Each female was kept with wild-type males for 48 hours. The males were then killed and the female was left in the same vial for three more days. At the end of that time, each female was transferred to a second vial and left for 24 hours. The female was then transferred to a third vial which contained *H/Me* males and was kept with those males for three days. After that period of time, the males were killed and the female was transferred to a fourth vial and kept in that vial for 24 hours. Counts were made of the offspring that each female produced in each of the four culture vials. The four vials represent, respectively, the first five days, the sixth, the seventh through the ninth, and the tenth day after the first mating was made. Offspring resulting from the mating with the first male should be normal (+), except for the glossy character in the males, and those from the second male should show one of the dominant markers *H* or *Me*.

RESULTS

The results of the tests for the period of reproduction of the wild-type and glossy females are shown in Table 1. Twenty wild-type and eleven glossy females were fertile. Wild-type females produced offspring throughout the nine days of the test and were more fecund than were glossy females. Except for one offspring that occurred on the fifth day after the mating, glossy females produced offspring only in the mating vials, shown as first day in Table 1, and in the vials containing eggs that were deposited on the second and third days after the matings were made. In contrast to the wild-type females, glossy females passed through a brief period of low fecundity and then became sterile. They did not regain their fecundity during the period of the test.

The breeding results with the females that were mated first to wild-type males and seven days later to *H/Me* males are given in Table 2. Offspring produced by the matings with the wild-type males are grouped under the column headed "Before Re-mating," which is subdivided into two columns; one showing the number of offspring produced in the first vial (first to fifth days of the test) and another giving the number of offspring

TABLE 1

PERIOD OF REPRODUCTION OF GLOSSY AND WILD-TYPE FEMALES FOLLOWING A SINGLE MATING

Days after mating		Number of offspring per day								
		1	2	3	4	5	6	7	8	9
Female type	Number fertile									
Wild	20	72	430	661	520	729	415	189	681	269
Glossy	11	7	51	22	0	1	0	0	0	0

produced in the second vial (on the sixth day after the mating). All of the offspring are expected to be normal, (+), with respect to *H* and *Me*. The offspring produced subsequent to the second matings are placed under the column marked "After Remating." The number and kinds of offspring produced for the first three days after the remating are given in the column headed "7th to 9th" days, the latter numbered days referring to the period of time after the first mating. Those offspring produced on the last day of the test are shown in the column marked "10th" day. The kinds of offspring occurring after the remating depend upon the sperm which were stored in the females. Those offspring that were due to sperm from the first males are recorded in the table as "+," and those from the second male are given as "*H–Me.*"

Although eight of the wild-type females produced progeny after the matings with both kinds of males, only seven of them still retained functioning sperm from the normal males at the time they were mated to the *H/Me* males. During the last four days of the test the seven females produced both "+" and *H–Me* offspring. The other female that produced offspring with both males had exhausted her supply of functioning sperm from the first male by the seventh day, producing only one "+" off-

TABLE 2

EFFECT OF REMATING ON FECUNDITY AS SHOWN BY THE NUMBER AND KINDS OF OFFSPRING PRODUCED BY WILD-TYPE AND GLOSSY FEMALES

		Before remating		After remating			
No. days after first mating		1st–5th	6th	7th–9th		10th	
Kinds of offspring		+	+	+	H–Me	+	H–Me
Female Type	No.						
Wild	7	547	136	220	77	31	72
	1	26	1	0	26	0	16
	3	168	47	114	0	50	0
	3	0	0	0	14	0	19
	1	4	0	0	0	0	6
Glossy	9	136	0	0	74	0	2
	16	212	0	0	0	0	0
	2	0	0	0	15	0	15
	38	0	0	0	0	0	0

spring on the sixth day, and produced only *H–Me* progeny during the seventh to tenth days. Three normal females were fecund throughout the test but produced no offspring with the second males. Three were sterile with the first males but were fertile after the remating. One female produced only four offspring and those occurred with the first male.

Twenty-five of the 65 tested glossy females were fertile with the first matings. All of them lost their fertility before the second matings were made and, consequently, produced no offspring in the second vial, on the sixth day of the test. After the remating, nine regained their fecundity. However, the second group of offspring were only *H–Me* and, therefore, due entirely to the sperm of the second males. The other sixteen that produced progeny with the first males and lost their fecundity failed to reproduce with the second matings. Two other glossy females produced offspring after the second mating but not with the first. Thirty-eight females were sterile to both matings.

Glossy and normal females differed in their response to the two matings. Normal females that were fertile with the first mating usually retained some of the first supply of sperm through the ten days of the test, and after the second mating the sperm from both males fertilized the eggs. Among the glossy females, 40 of the 65 that were tested produced no offspring with the first male. Even the 25 that were fertile lost their fecundity before the second matings were made. Sixteen of those females failed to reproduce with the second males, but the proportion of females sterile to the second mating is not appreciably higher than the proportion of those sterile to the first mating. The nine glossy females that reproduced with both kinds of males produced only *H–Me* offspring during the second period of fecundity. Yet the fact that they did produce the second group of offspring suggests that the eggs formed in the later period of life of the females can be fertilized.

DISCUSSION

Females homozygous for the mutant glossy have low fecundity as compared to wild-type females. Many of the glossy females produce no offspring, even though they have been inseminated. Even those which are not sterile produce their offspring only during the first 72 hours after mating.

The failure of glossy females to produce progeny after the third day is not due to any change in the eggs or to the lack of egg production. That the older females do produce eggs which are capable of being fertilized is shown by the results of a second mating which was made after the females had stopped reproducing (Table 2). Some of the females regained their fecundity when the second group of sperm was supplied. Although some of the females that were fertile with the first males were sterile with the second male, those that did reproduce with both sets of males were approximtely as fecund with the second as with the first males. The nine females produced 136 offspring during the first five days, a daily average of 27, and 74 during the first three days after the remating, a daily average of 25.

It seems probable that, as Oliver and Green have suggested, the loss of fecundity by glossy females is due to a change in motility or viability of the sperm that are stored in the ventral receptacle. Most of the wild-type females retained some of the first supply of sperm throughout the ten days of the test, and after the second mating the sperm from both kinds of males fertilized the eggs. However, none of the breeding glossy females had functioning sperm from the first males at the time the second males were added. After fecundity was reestablished by the second supply of sperm, all the offspring were *H–Me* and therefore due only to the sperm of the second males. Anderson and Oliver (1942) have examined glossy females for the presence of sperm in the genital tracts. They reported that movement of sperm can be observed in the ventral receptacle during the days immediately following matings but not in the older females. Sperm activity, therefore, closely parallels the drop in fecundity found in breeding tests.

A loss of sperm viability was found by Patterson, Stone and Griffin (1942) in their tests of *Drosophila virilis.* Miller (see Dobzhansky, 1941) reported that the sperm of *D. athabasca* retain their viability for a shorter period of time in *D. affinis* than in the receptacles of their own species. Glossy in *D. melanogaster* is a laboratory mutant which seems to have an effect on sperm viability in the tracts of the females within the species.

Although the general picture suggests markedly that the loss of sperm motility is the primary cause of decreased fertility in glossy females, the actual cause of sperm immobility remains unknown. Nonidez studied fertilization in *Drosophila* and suggested that the secretions of the parovarian glands might be necessary for the fertilization process. Other workers have suggested similar ideas (Gleichauf, 1936; Anderson and Oliver, 1942). However, new data that are to be reported by Anderson suggest that the spermathecae may be more important than the parovaria in affecting sperm motility.

SUMMARY

(1) Homozygous glossy and wild-type females were allowed to mate and then separated from the males. Wild-type females produced offspring through a test period of nine days, but glossy females became sterile by the fourth day and did not regain their fecundity during the period of the test.

(2) Wild-type females that were successfully mated to normal males, and seven days later, to *H/Me* males produced offspring after the second mating that were due to both males. The glossy females that were successfully mated to both kinds of males lost their fecundity before the second males were added and after the second mating produced offspring that were due only to the second males. The sperm stored in the ventral receptacles of the glossy females did not function as long as those in wild-type females, but a second supply of sperm again initiated fecundity in glossy females.

(3) It is concluded that since the eggs produced by glossy females after they have lost their fecundity can be fertilized, the loss of fecundity is not due to changes in the eggs or in egg production. The results support the conclusion that glossy females become sterile due to loss of motility or activity of the sperm that are stored in the ventral receptacle.

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NOTES ON GRAPHIC BIOMETRIC COMPARISON OF SAMPLES

A PAPER on biometric comparison of samples by Hubbs and Perlmutter[1] appeared while I was on active military service and only recently have I been able to study it. The procedure suggested by them, a modification of the graphic method of Dice and Leraas,[2] is valid and valuable. They do, however, raise two points that demand brief further discussion.

Hubbs and Perlmutter state that Simpson and Roe[3] claim that a different method of computing standard deviation between the differences of means "largely invalidates the Dice-Leraas method of comparing samples or calls for an involved estimate of the reliability of that method when the number of specimens (N) in the two samples is dissimilar." In fact, Dr. Roe and I stated that the Dice-Leraas "method is certainly valid in most cases." We pointed out, however, that the implicit criterion of significance is variable, that under some rather unusual but not negligible circumstances, which were carefully defined and adequately discussed, the results may be misleading, and that when doubt exists calculation of the statistical significance of the difference between means is advisable. What Hubbs and Perlmutter have contributed is, indeed, a method of estimating this significance for certain postulated sample relationships. In our book, previously cited, we gave the same method (in different and, for this particular application, less convenient form), and also methods applicable under other postulates. The apparent slight misunderstanding implied in Hubbs and Perlmutter's criticism of our views is unimportant except that it might arouse misgivings regarding the Dice-Leraas method by suggesting that there is some fundamental disagreement as to its validity under given conditions. The fact is that no such disagreement really exists.

Hubbs and Perlmutter also consider "unwise" our proposal that the values M ± 3σ be indicated on the Dice-Leraas graph, on the grounds that it "clutters the picture and adds little to its interpretive value" and that "extreme limits in the range of variation are hardly predictable in the variable characters of animals." But they have themselves equally cluttered the picture by adding the related values M ± 1σ to the graph, and their proposal seems to me to add distinctly less to the graph's interpretive value, for the average zoologist, than does ours. The purpose in each case is to give a measure of dispersion and to suggest the overlap of distributions in the populations represented. As M ± 3σ is both a measure of dispersion and an estimate of population range, it does give some idea of population overlap by a glance at the diagram, and this sort of quick graphic judgment is exactly what a diagram is for. The relation of M ± 1σ to population overlap is less clear and direct.

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[1] Am. Nat., 76: 582–592, 1942.

[2] Contr. Lab. Vert. Gen., Univ. Mich., 3: 1–3, 1936.

[3] "Quantitative Zoology." New York and London: McGraw-Hill Book Co. 1939.

It is true that the extreme limits of ranges of variation are not predictable in particular cases, but average limits can be estimated and the usefulness of such estimates needs no defense. If only subjectively, every zoologist does make such an estimate every time he compares samples. M ± 3σ is demonstrably a better estimate than is either an observed sample range or a subjective guess as to population range. As a statistical estimate of average tendencies, it is certain that this figure will sometimes be exceeded by the range in real populations and, less often and to smaller extent, in samples. Such cases are not exceptions in the usual sense, but are inherent in the nature of statistical estimation. The theory involved has the same basis as that of standard deviations and standard errors and if the variable biological factors stressed by Hubbs and Perlmutter in this connection were held to invalidate range estimates, they would also invalidate the other methods of comparison supported and proposed by those authors.

Further study has, indeed, shown that estimates of population range even better than M ± 3σ can be made. The defect of M ± 3σ is not that the principle (when correctly understood) is wrong, but that range varies with population size and this statistic is fully consistent only for natural populations of small size (about 450). Formal comparisons of extent of range and adequate studies of range overlap require that these be made on the basis of a conventional standard population size. I have elsewhere[4] discussed this important subject in some detail and have proposed a conventional standard range that is a more useful estimate than M ± 3σ. When it is desirable to suggest population dispersions and overlaps on a graph of Dice-Leraas type, inclusion of this standard range is recommended.

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[4] Simpson, *Amer. Jour. Sci.*, 239: 785–804, 1941. See also: Simpson and Roe, *Amer. Mus. Novitates*, No. 1190: 1–19, 1942.

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ONE- OR TWO-DIMENSIONAL ACTION OF MUTANT LOCI?

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IN a group of recent papers Curt Stern[1] analyzed genic action by studying the effects of a gene in different dosages, in different alleles and compounds and under different environmental conditions. It was believed impossible to explain the data which he obtained on the assumption of a single type of genic action. Stern, therefore, elaborated an ingenious though rather complicated scheme of genic action based on certain assumptions proposed by S. Wright. It is my intention to show that the factual data do not require such a complicated interpretation but fall, within expected limits, in line with formerly derived simpler assumptions.

A short summary of the decisive data in Stern's own words is:

> In *Drosophila* the fourth chromosome gene cubitus interruptus (ci) is recessive to wild type (+) at 25° and slightly dominant at 14°. At both temperatures the effect of two doses of ci is more normal than that of one and less than that of three. Thus ci contributes towards normality. The normal allele alone, in a single dose, assures normality at 25° and not at 14°, where a slight ci effect appears. At 14° the heterozygote +/ci deviates more from normality than flies carrying the +-allele alone: the cumulative effect due to "addition" of ci to a genotype containing ci only, is reversed into an antagonistic effect if ci is added to the +-genotype. Possession of both negative and positive properties of the ci allele may be interpreted, following Wright, in terms of (1) power of the gene to combine with a limited cellular substrate (S) and (2) efficiency of the gene in converting S into a product involved in the formation of normality. The ci-allele appears lower in both combining power and efficiency than the +-allele.

To these basic facts Stern and Schaeffer later added more data. (1) Three different wild-type alleles (in

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[1] C. Stern, *Genet.*, 28: 441–475, 1943. C. Stern and E. W. Schaeffer, *Proc. Nat. Acad. Sci.*, 29: 351–361 and 361–367, 1943.

Stern's interpretation) were found which acted differently in heterozygotes with ci. (2) The heterozygotes of these with a deficiency for ci (M_4) and the triplo-IV combinations of many viable combinations were added to the series. The results were explained again in terms of antagonistic effects based upon different gene-controlled reactions of the type just reported.

We begin with the three wild-type alleles which Stern calls $+^c$, $+^2$, $+^3$. If we call the interruption of the cubital vein in different degrees (Stern's classes 0–2) the ci effect, the first two "isoalleles" (Stern) have no effect at low temperature but $+^3$ has a slight ci effect, *i.e.*, a few ci individuals appear. Opposite a deficiency, however, all have a slight ci effect, which is largest for $+^3$ and intermediate for $+^2$. Though Stern describes these alleles as wild-type alleles we must not forget that in the usual terminology $+^3$ at least would be called a low ci-allele, just as vg^{nl} with no effect but a few nicked flies is called a vg-allele. This difference in terminology is of no importance in principle, for in such a case the limits between + and mutant alleles are arbitrary. But the issue might be confused if one does not realize that both sets of terms mean the same thing.

We turn now to the facts, *i.e.*, the phenotypes of different combinations, measured in ci classes N (= +) 0, 1, 2, of the alleles $+^c$, $+^2$, $+^3$, ci, Df ci (M_4), all in diploid and triploid (via triplo-IV) combinations. I have chosen to represent the facts graphically, using only a minimum of such assumptions without which genic action could not be described. We may assume that the effect of the loci is the production of vein-forming substance, whatever this may mean. (It would also be possible to work a reciprocal scheme with vein-preventing substance). We may also assume that the amount of this substance available at the time of determination of the cubitus is the consequence of a chain of reactions controlled by the loci in question. A definite amount surpasses the threshold above which the vein is normal; the abnormal classes are inversely proportional (*ceteris paribus, e.g.*, temperature, modifiers) to the amount of this substance below the threshold for normality. I repeat that these are the minimum assumptions without which no genic action can be described in dynamic terms. Thus we can represent Stern's data in the diagram Fig. 1 in terms of simple genic actions. The lines representing the reaction-chains of the different combinations studied by Stern are drawn so that they represent approximately (*i.e.*, by inspection of his data, not by exact calculation) the means of the effect. On the right of the figure actual variation as registered in Stern's tables is drawn half diagrammatically using his classification. This part of the diagram is to be superimposed upon the main drawing with lines t and t_1 being identical.

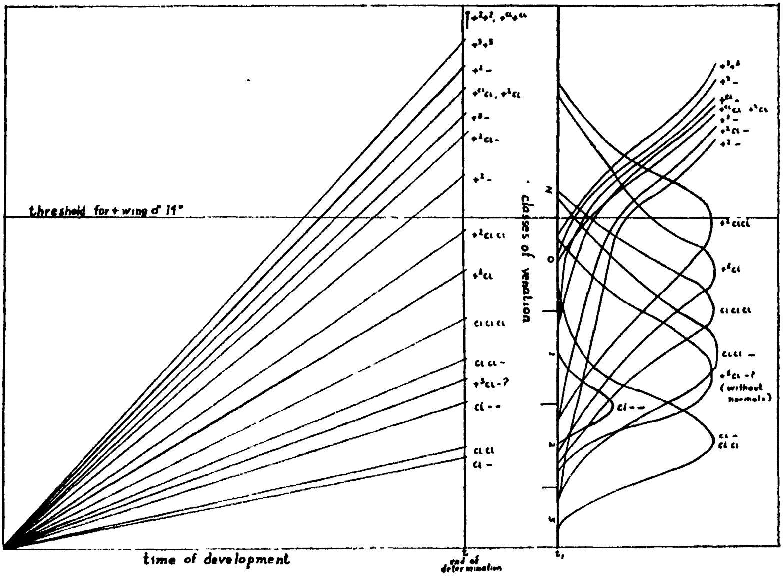

FIG. 1.

The basic data in this case as in many others require the assumption that there is a threshold for the determining reaction above which the normal wild type is produced. Any number of hyperthreshold wild phenotypes are imaginable, but they can be proven only if hemizygous or in compounds or if temperature or other effects push some individuals below the threshold. In these cases only the subthreshold part of the curves of variation is known (upper part of t_1), but the known part allows the allocation of the order of the means for hyperthreshold means. Means below the threshold are supposed to correspond to the means of the curves of variation of the effect and the measure are Stern's classes 0–3 as marked on t_1. The figure then represents the actual facts in terms of quantities of a single product of reaction.

Our problem is to find out whether these actual results are consistent without making further assumptions. This is identical with the question whether Stern's description of the facts is indispensable, namely, that ci in presence of ci only has a summative effect, adding towards normality, but in the presence of wild type has the opposite, antagonistic effect, subtracting from normality. It is this interpretation which requires, in Stern's view, a theory of the type he developed (see the quoted papers for this theory; not the theory itself but the need of its formulation is under discussion here).

In order to derive expectations on the basis of the simple description of genic actions in terms of quantities of reaction products we must look for a basis of our evaluation. The only visible one is found in the typical relations of multiple alleles. In the overwhelming number of instances the phenotype of a compound is intermediate between the two homozygotes, and therefore the action of the alleles n^1, n^2 in the compound is $\frac{n^1 + n^2}{2}$. If the wild type is completely dominant this may have the same cause, *i.e.*, the curve of the heterozygote +/n would be above the threshold. If dominance were the result of modifiers alone the system would still work in F_1. As in the present case two wild-type alleles are known which have subthreshold effects, (assuming Stern's terminology) the situation for these is the same as if they were lower multiple alleles of ci (which they are, in the usual way of describing such facts). Thus we may assume that the expectations for the phenotypes are always based upon the simple formula $\frac{n^1 + n^2}{2}$. There is no reason to expect a different effect in the case of one (deficiency) or of three (triplications) alleles, except for such actions based upon other loci involved, *i.e.*, within the deficiency or within the duplication (triplo IV). Thus a first approximation to our expectations for the phenotypes must be derived from the known differences of the homozygotes, using the formula $\frac{n^1 + n^2 (+ n^2)}{2}$ for the different combinations. We know in the present case the order of effects of $+^c$, $+^2$, $+^3$ homozygous and of ci ci. If we assume a value for the threshold of normalcy (mean) of 20 units of the determining principle we can assign the following mean estimated values: $+^c = 44$, $+^2 = 34$, $+^3 = 24$, assuming that the normal variation (at 25°) does not transgress the threshold at 20; ci would have an action of 8 units. For the deficiency we do not have much information; its action value might be zero or a small value. We will assume a value of 2. Thus we can calculate the phenotypic expectation for all combinations in relation to the basic homozygous phenotypes and establish the expected order of phenotypes on the basis of effects of simple proportions. This has been done in Table 1, where the first three columns give the expected order and the fourth the actual order as found in Fig. 1. Curves which are near each other have been given the same number. It is needless to say that this procedure is a rather crude one. But as we have no way of knowing whether a small but statistically significant difference in the mean is exclusively due to the specific genic action, more refined methods are hardly needed.

Looking at the table we see that with only two or three major exceptions the results are roughly those expected on the basis of a simple genic action. In all the smaller discrepancies the deficiency is involved which is known to affect general developmental features. Only one combination falls completely out of the series, namely $+^2$ ci ci, where the two ci are largely dominant. But this result can not be derived from Stern's antagonistic actions either: his viewpoint would account for this as well as the opposite result, if the latter would have been found (as it is found in a majority of triploid combinations of other loci). The No. 9 case with a shift out of order towards ci is hardly in agreement with Stern's interpretation except if a special assumption is made regarding the action of the deficiency. But this combination is not completely reliable (see footnote); in case No. 11, the third aberrant case, the deficiency acts in the opposite direction. I find difficulties in applying the same interpretation to both cases. Further Nos. 7–8 and 10–11 ought to give parallel results on whatever assumption, but their order is in opposite directions. I am inclined to consider this as based on chance (which includes the action of deficiencies) rather than upon such differences between $+^c$ and $+^3$ which Stern must assume to explain the small discrepancy.

TABLE 1

Expected order	Compound	Value $\frac{n^1 + n^2 (+ n)}{2}$	Actual order ♂ cold	Far off towards + or ci
1	$+^c +^c$	44	1	
2	$+^2 +^2$	34	2	
3	$+^2$ ci ci	25	8	! → ci
4	$+^3 +^3$	24	3	
5	$+^c$ – (– = DfM$_4$)	23	4	
6	$+^2$ ci –	22	4	
7	$+^2$ ci	21	5	
8	$+^2$ –	18	5	
9	$+^3$ ci –	17	8*	! → ci
10	$+^3$ ci	16	7	
11	$+^3$ –	13	6	! → +
12	ci ci ci	12	9	
13	ci ci –	9	10	
14	ci ci	8	11	
15	ci – –	6	10	
16	ci –	5	12	

* This does not follow directly from Stern-Schaeffer's data in which the N-class could not be checked but is extrapolated from the trend of the curve in the observed classes.

Looking over the entire assemblage of data I come to the conclusion that with one exception (+ ci ci) the facts do not require the assumption of two opposite effects of ci, whether alone or combined with plus. I wonder whether the small discrepancies from simple expectations require the complicated theory which is based upon the description of the data in terms of antagonistic effects. I wonder further whether in the majority of cases in which the +rr (r = recessive) form is known to be of the wild type the dosage rule for rrr does not hold. I might finally venture the opinion that a simple explanation would not have appeared so difficult if Stern had not interpreted the dosage effects of the series ci, ci ci, ci ci ci as demonstrating that ci contributes something towards normal. This seems to me to be an unnecessary method of interpretation when the "naive" interpretation expressed in the formula $\frac{\text{ci} + \text{ci} + \text{ci}}{2}$, etc., describes the facts perfectly.

Speaking generally, then, the difference between our views is this: Stern attributes the decisive weight to such discrepancies within comparable pairs as the inverted order of effects in Nos. 10–11 or 9–11, discarding the possibility of specific influences of the deficiencies upon the normal rate of the underlying processes. I prefer to look at the general orderliness of the whole series and to attribute minor discrepancies to such specific features as the ontogenetic effects of deficiencies. The only really great discrepancy, No. 3 of Table 1, is the case in which considerable dominance of two recessives over one dominant is involved. I am inclined to consider this as a special phenomenon to be interpreted independently of the simple proportionality of actions of the other combinations. Here the problem of the "covering or not covering" of recessives by a duplication enters, which, I think, has not yet found a solution.

The reason why this criticism is offered is first that Stern's experiments are so excellently conceived and carried out that their interpretation is of paramount importance; second, that the present author strongly believes that simple explanations ought to be preferred as long as it can possibly be justified, because organic processes could never work if they were not basically simple. I am greatly indebted to my friend Professor Stern for reading the manuscript of this paper, stating his counter criticism and assenting to its publication.

CHROMOSOME STUDIES ON EIGHT SPECIES OF SCIARA (DIPTERA) WITH SPECIAL REFERENCE TO CHROMOSOME CHANGES OF EVOLUTIONARY SIGNIFICANCE[1]

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INTRODUCTION

WITH the rediscovery of the giant chromosomes in the larval salivary glands of the Diptera by Heitz and Bauer (1933) and their application to genetic research by Painter (1934), students of evolution were provided with a new and powerful tool for studying the problems of evolutionary chromosome changes. In *Drosophila,* numerous investigators have made great progress in analyzing and interpreting naturally occurring salivary gland chromosome changes, and in comparing them with similar ones induced in the laboratory. The question of the applicability of the results reported for *Drosophila* to biological material in general is one of great moment and one which can only be answered by making comparable studies on other material.

Next to *Drosophila,* the best known genus from this standpoint is *Sciara* (Mycetophilidae). This generic group is particularly favorable for comparison with *Drosophila* because it is taxonomically far removed from the latter and because its chromosomes are equally favorable for study.

As is now well known, each giant salivary gland "chromosome" consists of a pair of intimately synapsed homologues, and has its own particular pattern of cross bands or discs. Thus, microscopically visible chromosome alterations are readily detected in heterozygous individuals by examination of the disc patterns. The method has been used for study of both naturally occurring alterations and artificially induced changes. For a review see "Genetics and the Origin of Species" (Dobzhansky, 1941).

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[1] Dissertation presented to the Faculty of the Graduate School of the University of Pennsylvania in partial fulfillment of the requirements for the doctor's degree from the Department of Zoology.

In *Drosophila,* studies have established the following types of chromosome aberrations (changes): (1) inversions of one or more blocks of bands, (2) translocations of regions within the same chromosome and between chromosomes, (3) "repeats" or duplications of small regions, and (4) deletions of one or more bands.

In studies of *Sciara ocellaris* Comst., and of hybrids between this species and *S. reynoldsi* Metz, Metz (1938a) and Metz and Lawrence (1938) found evidence of considerable difference between *Sciara* and *Drosophila* with respect to chromosome changes occurring in nature.

In *Drosophila,* inversions are common among the different strains of the same species, and species hybrids usually differ from each other by numerous inversions. Minute changes in the salivary chromosomes, such as alterations in the appearance of single discs or small clusters of discs, are relatively rare but have been described in several hybrids, among them *D. melanogaster* by *D. simulans* (Kerkis, 1937, Horton, 1939).

The studies by Metz and Metz and Lawrence cited above on hybrids between *Sciara ocellaris* and *S. reynoldsi* showed no evidence of major chromosome inversions. Minute changes were readily found in different strains of *S. ocellaris* and seemed to be the main mode of differentiation between the hybrid chromosomes studied.

The present investigation represents an extension of the *Sciara* studies designed to ascertain whether or not conditions are the same in different species of this genus and whether there is general indication that the genus as a whole differs from *Drosophila* with respect to evolutionary chromosome changes.

It has been found that small differences involving only one or a small cluster of discs do seem to be characteristic of the genus *Sciara,* as distinguished from *Drosophila,* but that the findings of Metz (1938a) and Metz and

Lawrence (1938), which indicate an absence of large chromosome rearrangements, do not apply to the genus as a whole.

The present investigation has revealed a general tendency in *Sciara* for "repeats" to be associated with the X chromosome. Such a phenomenon has not been encountered in *Drosophila* and at present remains without interpretation in *Sciara.* In addition to the study of the salivary gland chromosomes, other observations, described below, were made in order to supplement those of earlier observers.

The sex ratios in the genus *Sciara* were found by Metz (1925, 1926b, 1938b) to be determined by the mother irrespective of the male and were found to vary from the common 1:1 ratio. In some species, *e.g., Sciara coprophila,* individual females produce unisexual progenies; *i.e.,* either males or females; in other species, *e.g., Sciara prolifica,* individual females regularly produce bisexual progenies. Hence, the kind of progeny produced by each of the species studied in this paper has been recorded with interest.

In this paper the term *progeny* will be used to refer to all of the offspring from any individual female. Species in which the individual females produce offspring of only one sex, either male or female, will be said to produce unisexual progenies. Species in which the individual females regularly produce both males and females will be said to produce bisexual progenies. It is important to note that females of species which produce unisexual progenies do occasionally produce "exceptional" individuals of the opposite sex. However, no attempt has been made to check the frequency of occurrence of these "exceptional" individuals. Further it should be noted that sex ratios were not recorded for the species discussed in this paper which produce bisexual progenies.

In addition to the group of "ordinary" specific chromosomes, earlier studies in *Sciara* revealed different numbers of "limited" chromosomes, depending on the species (see Metz, 1938b, for a review of the conditions in *Sciara*). "Limited" chromosomes are usually larger than the others and appear to have a greater diameter. Although these chromosomes are apparently genetically inert or nearly so, they reproduce and go through the mitotic process with the other chromosomes. The "limited" chromosomes number from one to three in those species which possess them and are restricted to the germ line. They are eliminated from the soma at either the fifth or the sixth cleavage division of the egg (Dubois, 1933).

Since the significance of the "limited" chromosomes is still obscure, attention has been directed in this investigation toward determining their presence or absence, their numbers and their morphological character for each of the eight species studied.

Metz and Moses (1923) have shown in a survey of the somatic chromosome groups of a number of species of *Drosophila* that the different species have different chromosome numbers. Nearly all the variations, however, could be explained by assuming appropriate rearrangements of the chromatin of the basic number of four chromosome pairs, *e.g.*, fusion of rods to form V's, conversion of V's to rods, etc. It seems probable that these rearrangements have been significant in the evolutionary process (see Sturtevant and Novitsky, 1942). A similar study of the somatic chromosome groups of the eight species of *Sciara* reported here has been made to determine whether or not similar forces affecting chromosome rearrangements are operating within this genus.

The author is indebted to Dr. Metz for suggesting the problems investigated, for his help in carrying them out and for his advice and help in the preparation of this manuscript.

MATERIALS AND METHODS

1. ESTABLISHMENT AND IDENTIFICATION OF CULTURES

The stocks of the several species of *Sciara* studied were all established in the same manner. Collecting trips to a number of greenhouses were made wherein single gravid females were trapped on the glass by covering them with small vials partially filled with agar. The vials with the single females were returned to the laboratory and kept at room temperature to await the laying and hatching of eggs. Subsequent generations were reared according to the procedure outlined by Smith-Stocking (1936).

About a thousand females were captured, representing about thirty different species of which only eight gave rise to laboratory stocks. The different species were designated with Roman numerals and collections of each, composed of pinned specimens, alcoholic specimens, wing mounts and hypopygial mounts, were made. The author wishes to thank Dr. Frank R. Shaw for examining some of the material and identifying species V as *Sciara nacta* Johannsen, and Dr. Alan Stone for his advice and kindness in examining specimens of each species and identifying species IV and VI as taxonomically the same, being *Sciara fenestralis* Zetterstedt; species XV–6 as *Sciara prolifica* Felt, Johannsen's variety b; and species XXIV as *Sciara agraria* Felt. The other species, namely VII, XXIII and XXV, Dr. Stone reports as undescribed; hence, they will be referred to by Roman numerals throughout this paper.

Although IV and VI are morphologically similar and have been called the same species by Dr. Stone, the author believes them to be distinct species and will treat them as such for the following reasons:

a. Examinations of wing mounts of the two forms show the petiole of the cubitus to be about .8 that of the basal section of the media in IV and only .5 in VI.

b. Species IV females are "bisexual producers"; *i.e.*, individual females of this form *regularly* produce both males and females, with males in excess. In contrast, species VI females are *regularly "unisexual producers"; i.e.*, individual females of this form regularly produce one sex, either males or females. Exceptional individuals occur in both types of progenies.

c. Reciprocal crosses failed to produce hybrids between these forms.

d. Banding patterns of the salivary gland chromosomes do not match in the case of the X chromosomes, nor do they match in the cases of the other similar length chromosomes.

e. Other cases such as this are on record; *e.g.*, Dobzhansky's *Drosophila pseudoobscura* a and b (Tan, 1935), or Metz's *Sciara ocellaris-reynoldsi* case (Metz and Lawrence, 1938).

Hence, I shall refer to IV as *Sciara fenestralis* form I and to VI as *S. fenestralis* form II.

2. GONIAL METAPHASE FIGURES

Gonial metaphase figures were obtained by dissecting out the gonads of larvae two to five days after hatching. The dissection was made in Ringer's solution. The excess Ringer's was drawn off and a small drop, 3 mm in diameter, of acetocarmine added. Immediately the gland was smeared by dropping a cover slip on it. The edges of the cover slip were ringed with vaseline and the preparation allowed to stand for twelve hours in a moist container. The preparation was made permanent by the following procedure: the vaseline was removed and the slide placed cover slip down supported by two glass rods in a petri dish containing a mixture of equal parts of glacial acetic acid, 95 per cent. alcohol and clove oil; when the cover slip had floated off, either the slip or the slide, to whichever the gland adhered, was washed in two changes of 95 per cent. alcohol after which it was mounted in diaphane.

3. SOMATIC METAPHASE GROUPS FROM CEPHALIC GANGLIA AND SALIVARY GLAND PREPARATIONS

Cephalic ganglia and salivary glands were dissected out in body fluid and immediately transferred to a small drop, 3 mm in diameter, of 45 per cent. acetic acid on a clean glass slide. The glands and ganglia were smeared by dropping a cover slip on them. The preparation was placed in 95 per cent. alcohol vapor for three hours; then the cover slip was soaked off in 95 per cent. alcohol. The slip or slide bearing the material was returned to 45 per cent. acetic acid for one minute, thence into acetocarmine for five minutes. The stained preparation was rinsed in 95 per cent. alcohol, passed quickly through a 45 per cent. acetic acid bath to remove excess stain, dehydrated 5 minutes in 95 per cent. alcohol, and mounted in diaphane.

The somatic chromosome groups show that all species studied have the same diploid chromosome number, namely, eight. The shapes and sizes of the chromosomes, however, differ in different species.

The gonial chromosome groups show, in some cases, chromosomes which have no homologues in the soma. These chromosomes have been referred to in the introduction as "limited" chromosomes. Like the "ordinary" chromosomes, these, too, differ in size and shape. Particular attention should be called to the fact that so far as observed those species having rod-shaped "limited" chromosomes have two of them. This point will be emphasized in the discussion.

For each of the eight species studied, both ends of each of the salivary gland chromosomes have been mapped in addition to regions of special interest, such as asynaptic regions, repeated regions, etc. In discussing the salivary gland chromosomes of each species studied, the longest autosomal chromosome in each complex will be referred to as the A chromosome, the next longest as the B and the shortest as the C. This grouping does not imply that all A chromosomes, for instance, are homologous; but merely that each represents a similar proportionate length with respect to the other chromosomes in each of the several species complexes.

The findings with respect to the features mentioned above will be presented for each species separately, together with information concerning the source of material, distribution of the species when obtained in more than one place and any special remarks.

OBSERVATIONS

Sciara agraria Felt

1. SOURCE AND GENERAL CHARACTERISTICS

The laboratory stock from which the following data were obtained was established by making brother-sister matings from the offspring of one of several females collected from a flower pot in a residence in Telford, Pa.

This species grows well in the laboratory. It has been secured in two other localities within a forty-mile radius of Telford. Individual mated females of this species produce bisexual progenies.

2. SOMATIC AND GONIAL METAPHASE CHROMOSOMES

The somatic chromosome complex (Fig. 6b) shows two pairs of V-shaped chromosomes and two pairs of rod-shaped chromosomes. The gonial metaphase chromosome group (Fig. 6a) shows three pairs of V's and two pairs of rods. One pair of V's is decidedly larger than the other two pairs. The large V-shaped chromosomes with no counterparts in the soma are the "limited" chromosomes. The "limited" chromosomes are eliminated from the soma at an early cleavage division, as mentioned above.

3. SALIVARY GLAND CHROMOSOMES

The salivary gland chromosomes in this species spread easily and stain well; hence, they offer no technical difficulties. Figs. 9a and 9b show the two ends of the C chromosome. The two ends of the A chromosome are shown in Figs. 11a and 11b. There were no irregularities found in these two chromosomes.

Small differences between homologues. Figs. 12a and 12b represent the two ends of the B chromosome. The arrow in Fig. 12a indicates a small difference in banding pattern. Either one band in one homologue is missing or an extra one is present in the other. In this case, the extra band in the one homologue was sufficient to cause a small asynaptic gap between the two homologues.

"Repeats." Fig. 10a shows one end of the X chromosome in which there has been a duplication of a short region of the chromosome. These two homologous portions of the chromosome have synapsed to form a short, tight loop. Fig. 10b shows the other end of the X chromosome.

Rearrangements. In the material examined representing this species, no large rearrangements were found, but not enough specimens were secured to show that such rearrangements do not occur in the species.

Sciara species XXV

1. SOURCE AND GENERAL CHARACTERISTICS

Several females of this species were collected in a greenhouse in Norwood, Pa. The species thrives in the laboratory and mated females produce bisexual progenies in which neither sex occurs in great excess of the other. This species was found only once and is undescribed.

2. SOMATIC AND GONIAL METAPHASE CHROMOSOMES

The somatic metaphase chromosome group (Fig. 5b), like the gonial metaphase chromosome group (Fig. 5a), shows one pair of V-shaped chromosomes and three pairs of rod-shaped chromosomes. This species, according to this material, possesses no "limited" chromosomes, a characteristic wherein it resembles *Sciara ocellaris* and *S. reynoldsi.* Metz and his students have studied the latter two species extensively and have not yet found a race which possesses "limited" chromosomes. Hence, it is likely that the "limited" chromosomes have been lost from these species.

3. SALIVARY GLAND CHROMOSOMES

The ends of the salivary gland chromosomes in this species are sufficiently different from each other so that no difficulty is experienced in differentiating between them. The two ends of the A chromosome are seen in Figs. 14a and 14b. Figs. 15a and 15b represent the two ends of the C chromosome. Figs. 16a and 16b show the two ends of the B chromosome.

Rearrangements and small differences between homologues. In this particular stock no small differences and no large chromosome rearrangements were observed. But as has been mentioned above for a similar case, this does not necessarily mean that these phenomena do not occur in this species.

"Repeats." One end of the X chromosome (Fig. 13a) shows two similar regions of at least three bands which are not tightly synapsed in this figure but are spread apart, remaining connected by several heterochromatic strands. In most of the cells examined, this repeated region was more tightly synapsed, allowing but a very limited view of the duplicated region. The banding pattern of the other end of the X chromosome is shown in Fig. 13b.

Sciara prolifica Felt Johannsen's var. b

1. SOURCE AND GENERAL CHARACTERISTICS

The laboratory stock of *S. prolifica* from which these observations were recorded was started from brother-sister matings of the offspring of a single female which was collected in a greenhouse in Johnson City, Tenn. Several females were collected at the same time in the same greenhouse. However, material from only one of them was used in building up a laboratory stock. Mated females of this species produce bisexual progenies. This species has been found in nearly every greenhouse examined from New York to South Carolina where horse manure is used. In addition to the greenhouse collections, this species was obtained in great numbers around a pile of chicken manure, in an open meadow in Ridley Park, Pa.

In addition to preparations made from the stock which was selected as a type, a few preparations were made from offspring of four different collections. The Tennessee stock was maintained because it contained both a "repeat" and a chromosome rearrangement, whereas the other stocks had only the "repeat." To investigate the potentialities of these various stocks by cross breeding was not thought profitable, since Mr. Carson was making such a comprehensive study for *Sciara impatiens.*

2. SOMATIC AND GONIAL METAPHASE CHROMOSOMES

The somatic metaphase chromosome group (Fig. 8b) is composed of two pairs of V-shaped chromosomes and two pairs of rod-shaped chromosomes. The gonial metaphase group (Fig. 8a) shows one large V-shaped chromosome in addition to those chromosomes which are homologous to the chromosomes in the somatic metaphase. This large V-shaped chromosome is the "limited" chromosome.

3. SALIVARY GLAND CHROMOSOMES

The salivary gland chromosomes of this species are quite different in appearance from those of the other species examined. They are short, thick and grossly resemble short pieces of rope. They separate from each other readily when smeared, and stain easily. However, it is difficult to obtain the larvae in the stage in which the stained bands of the salivary chromosomes are sharply delimited from each other. Figs. 18a and 18b show the two ends of the A chromosome. Figs. 20a and 20b show the two ends of the B chromosome. The distal tip of the B chromosome (Fig. 20b) is doubled back on itself. This was a characteristic of this end of the B chromosome which manifested itself in different ways. In some preparations this tip was pulled away from the rest of the chromosome, maintaining only a thin heterochromatic strand connection.

Small differences between homologues. No small differences were found in the Tennessee stock, which was studied intensively. Only a limited study was made of the ends in the other stocks; these did not show any small differences. Thus, evidence is not conclusive as to the occurrence of minute changes in this species.

"Repeats." Fig. 19a shows one end of the X chromosome. Fig. 19b shows the mid-region of the X chromosome. The small check marks opposite the bands in Figs. 19a and 19b indicate that these are the same bands. Fig. 19c represents the other end of the X chromosome. The two ends (Figs. 19a, 19c) were drawn from a different cell than was the mid-region of the X chromosome (Fig. 19b). The mid-region of this chromosome shows a double looped configuration. This was caused by the

synapsis of the three similarly banded regions, the pattern of which was not clear in any slide. This is an example of a double "repeat."

Rearrangements. Fig. 17 shows the C chromosome in which there has been an inversion involving about three fourths of the length of this chromosome. This figure, as well as all others of this inversion, indicates this to be a terminal inversion; that is, one involving the entire distal tip of the chromosome. Slides of three of the four other stocks did not show this inversion. The slides of the fourth stock showed the inversion, but the banding pattern was not good enough to admit of analysis.

Sciara species XXIII

1. SOURCE AND GENERAL CHARACTERISTICS

This species was taken independently by two different collectors in greenhouses in Wilmington, N. C.; Columbia, S. C.; and Charleston, S. C. Offspring from one of the females collected in Wilmington provided the sibs used in establishing the laboratory stock from which the following data have been secured. A few slides were made from the F_1 larvae of the females collected at the other two cities also. This species gives rise to bisexual progenies.

2. SOMATIC AND GONIAL METAPHASE CHROMOSOMES

The somatic chromosome group (Fig. 3b) shows a variation in the shape of the chromosomes. There are no V-shaped chromosomes present, but four pairs of rods. The gonial group (Fig. 3a), like the somatic group, has all rod-shaped chromosomes. In addition to those chromosomes which are homologous to those in the soma, there are two large, unpaired, rod-shaped chromosomes. These are the "limited" chromosomes.

3. SALIVARY GLAND CHROMOSOMES

The salivary gland chromosomes of this species seemed to be exceptionally long. Many slides showed cells in which the four chromosomes were joined together, the joined ends forming a hub from which the chromosomes radiated. Examination of those cells in which the chromosomes were joined and those in which the chromosomes were not joined showed that (a) the same chromosome ends were always united in the joined state and (b) in the unjoined state the ends homologous to the uniting ends were often ragged with heterochromatic material protruding from them. Fig. 23a shows such a condition in end 1 of the C chromosome. This behavior of the salivary chromosomes is similar to that found in *Sciara reynoldsi* and is suggestive of the chromocenter condition which is found in *Drosophila.* Fig. 23b shows the other end of the C chromosome. Fig. 21a shows the banding pattern of end 1 of the A chromosome. Although this chromosome end is broken in this figure, all the bands are present. End 2 of the A chromosome is seen in Fig 21b.

Small differences between homologues. One small difference was found is several cells from one individual in this species. This is shown in Fig. 22a, which is end 1 of chromosome B. The arrow in this figure indicates a thin band opposed to a thick band. Fig. 22b represents end 2 of the B chromosome.

"Repeats." The X chromosome in this species has a single duplicated region. When these two homologous regions synapse, a short looped figure is produced (Fig. 24b). This is a typical "repeat" figure in which the homologous regions have become linearly separated. As in most of these "repeats," the banding pattern is confused and determining how many bands are involved is difficult. Fig. 24a shows end 1 of the X chromosome. This figure, like 23a, shows the sticky attenuated condition of the end which is in many cases joined to the ends of the other chromosomes. Fig. 24c represents end 2 of the X chromosome.

Rearrangements. No large rearrangements were found in any of the three stocks of this species examined. Again, however, there is insufficient evidence to say that gross rearrangements do not occur in this species.

(*To be concluded*)

EXPLANATION OF PLATES

Figures on Plate I are camera lucida drawings at a magnification of 2,100 diameters. Figures on Plates II, III, IV and V are camera lucida drawings, all at the same magnification as indicated by the scale on each plate. Figures on Plate VI are photomicrographs. Fig. 45 was photographed at the magnification indicated by a scale just under it; all other figures are at the magnification indicated by a scale above Fig. 41. All figures on Plate VII are outline drawings, drawn with the aid of a camera lucida. The magnification scale beneath each figure represents .1 mm.

EXPLANATION OF FIGURES, PLATE I

1a, 1b, *Species VII.* Gonial metaphase group (4 pairs of rods—1 pair of V's), somatic metaphase group (3 pairs of rods--1 pair of V's). "Limited" chromosomes in 1a are indistinguishable.

2a, 2b, *Sciara nacta.* Gonial metaphase group (5 pairs of rods), somatic metaphase group (4 pairs of rods). Difficult to distinguish "limited" chromosomes, probably the two largest rods in 2a.

3a, 3b, *Species XXIII.* Gonial metaphase group (5 pairs of rods), somatic metaphase group (4 pairs of rods). Difficult to distinguish "limited" chromosomes.

4a, 4b, *Sciara fenestralis,* form I. Gonial metaphase group, (2 pairs of rods—3 pairs of V's), somatic metaphase group (2 pairs of rods—2 pairs of V's). The large V-shaped chromosomes at the bottom of figure 4a are the "limited" chromosomes.

5a, 5b, *Species XXV.* Gonial and somatic metaphase groups (3 pairs of rods—1 pair of V's). No "limited" chromosomes.

6a, 6b, *Sciara agraria.* Gonial metaphase group (2 pairs of rods—3 pairs of V's), somatic metaphase group (2 pairs of rods—2 pairs of V's). The two large chromosomes in the lower left hand corner of figure 6a are the "limited" chromosomes.

7a, 7b, *Sciara fenestralis,* form II. Gonial metaphase group (2 pairs of rods—2 pairs of V's—1 single large V), somatic metaphase group (2 pairs of rods—2 pairs of V's). Large single V in 7a is the "limited" chromosome.

8a, 8b, *Sciara prolifica,* Johannsen's var. b. Gonial metaphase group (2 pairs of rods—2 pairs of V's—1 large single V), somatic metaphase group (2 pairs of rods—2 pairs of V's). Large single V in 8a is the "limited" chromosome.

EXPLANATION OF FIGURES, PLATE II

9-12 *Sciara agraria.* Salivary gland chromosomes.

9a End 1 of "C" chromosome.

9b End 2 of "C" chromosome.

10a End 1 of "X" chromosome with "repeat" region shown about one third the length of the chromosome from end 1.

10b End 2 of "X" chromosome.

11a End 1 of "A" chromosome.

11b End 2 of "A" chromosome.

12a End 1 of "B" chromosome. Arrow in this figure indicates a small difference in banding pattern in which one band in one homologue is missing.

12b End 2 of "B" chromosome.

13–16 *Sciara species XXV.* Salivary gland chromosomes.

13a End 1 and mid-region of "X" chromosome including "repeat" regions which have been pulled apart leaving a thin strand between them.

13b End 2 of "X" chromosome.

14a End 1 of "A" chromosome.

14b End 2 of "A" chromosome.

15a End 1 of "C" chromosome.

15b End 2 of "C" chromosome.

16a End 1 of "B" chromosome.

16b End 2 of "B" chromosome.

EXPLANATION OF FIGURES, PLATE III

17–20 *Sciara prolifica* (Felt), Johannsen's var. b. Salivary gland chromosomes.

17 Terminal inversion of "C" chromosome involving two thirds of the length of the chromosome.

18a End 1 of "A" chromosome.

18b End 2 of "A" chromosome.

19a End 1 of "X" chromosome.

19b Extended regions of "X" chromosome showing "double repeat" in which the three homologous regions are synapsed. The check mark opposite the dark band of 19a and that opposite the dark band in figure 19b indicate that the two bands are the same.

19c End 2 of "X" chromosome.

20a End 1 of "B" chromosome.

20b End 2 of "B" chromosome.

21–24 *Sciara species XXIII.* Salivary gland chromosomes.

21a End 1 of "A" chromosome.

21b End 2 of "A" chromosome.

22a End 1 of "B" chromosome. Arrow in this figure shows a thick band in one homologue apposed to a thin band in the other.

22b End 2 of "B" chromosome.

23a End 1 of "C" chromosome. "Sticky" condition of the end indicated by the material extending from the vesicle. The same sort of condition is shown in 24a.

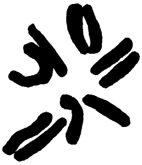
23b End 2 of "C" chromosome.

24a End 1 of "X" chromosome.

24b Mid-region of "X" chromosome showing synapsis of two homologous regions ("repeat").

24c End 2 of "X" chromosome.

PLATE I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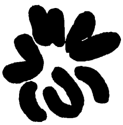
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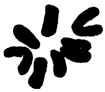
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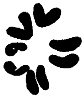
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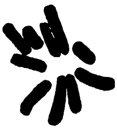
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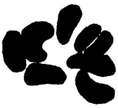
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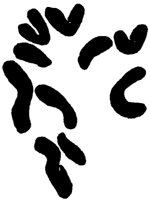
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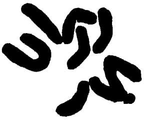
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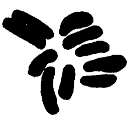
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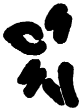
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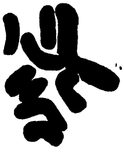
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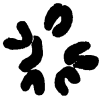
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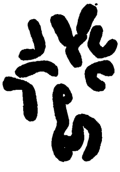
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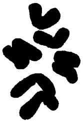
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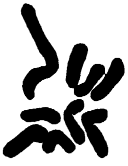
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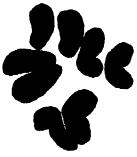
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PLATE II

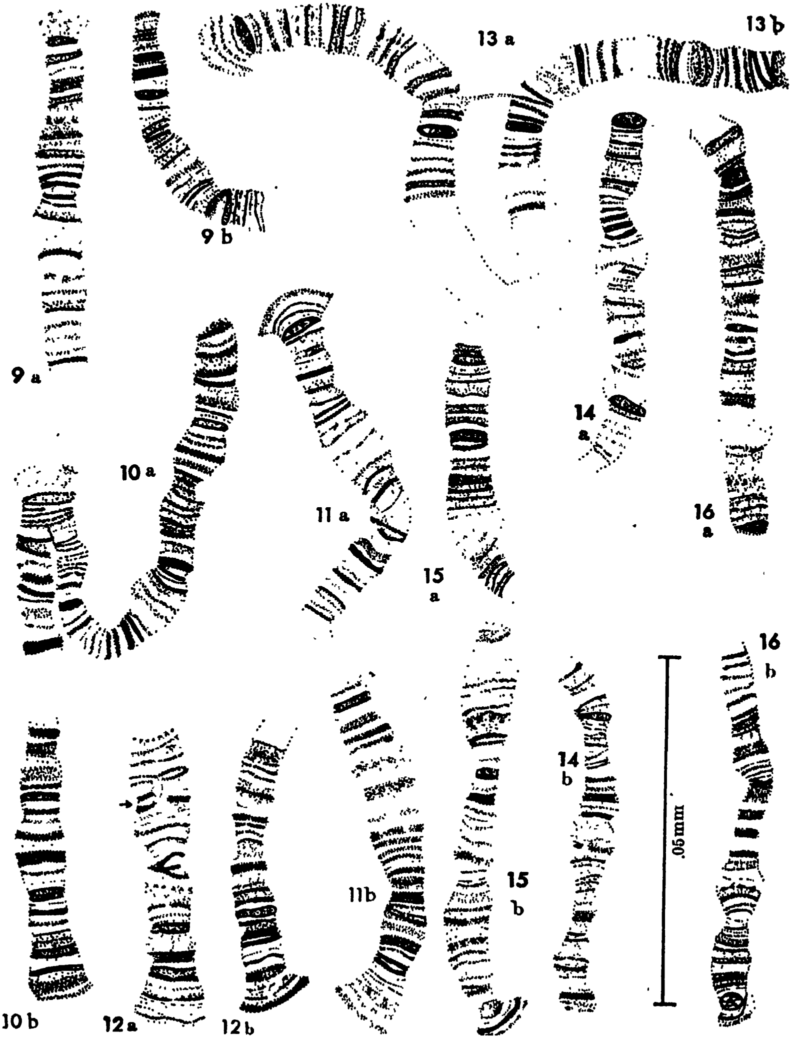
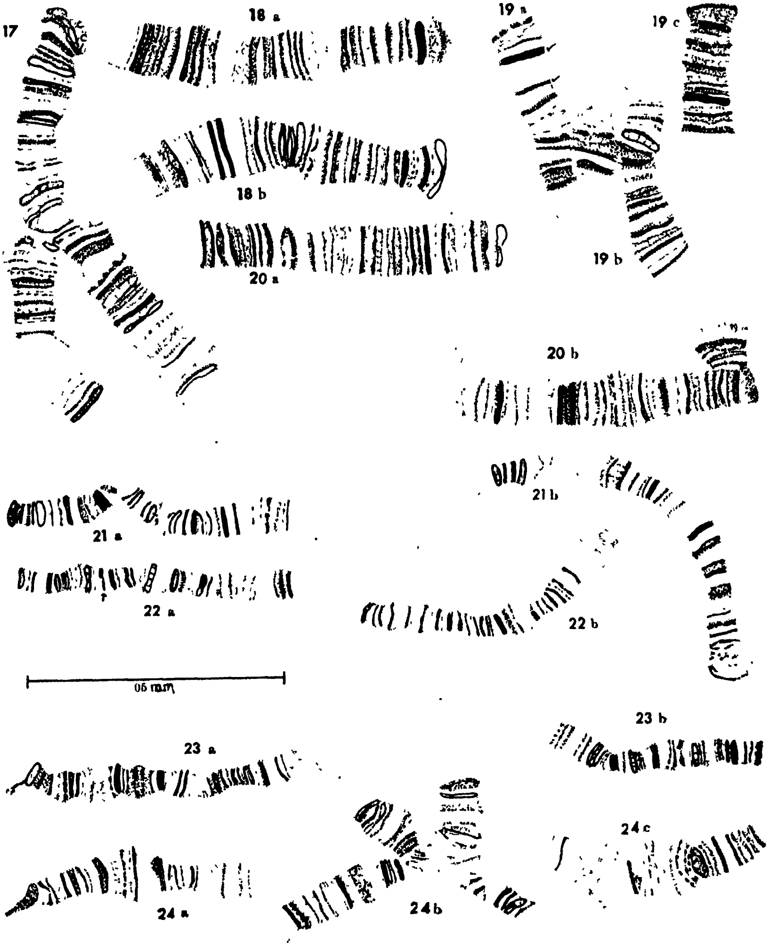

9 a
9 b
13 a
13 b
10 a
11 a
15 a
14 a
16 a
16 b
14 b
.05 mm
15 b
11b
10 b
12 a
12 b

PLATE III


17
18 a
18 b
19 a
19 c
19 b
20 a
20 b
21 a
21 b
22 a
22 b
05 mm
23 a
23 b
24 a
24 b
24 c

THE BISEXUALITY OF UNIPARENTAL HYMENOPTERA, A FUNCTION OF THE ENVIRONMENT[1]

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REPRODUCTION in the Hymenoptera is characterized by the production of uniparental apomictic[2] individuals. The normal male, always uniparental, is haploid. The female, either uniparental or biparental, is diploid.

In a uniparental species, the progeny of unmated females are usually females; in a biparental species, usually males. There is, however, in many if not all hymenopterous species, a capacity to produce both sexes uniparentally (amphitoky). This uniparental bisexuality, as recorded for a number of hymenopterous species, appears to be an effect of environmental factors.

The purpose of this paper is to present evidence supporting the thesis that the sexuality of a uniparental population is an expression of the type of germinal tissue in the preceding generation, the type being determined by environmental conditions. The observations forming the basis of this study were made during the mass propagation of beneficial insects at the University of California Citrus Experiment Station, Riverside, California.

THE PREVALENCE OF UNIPARENTAL REPRODUCTION IN THE HYMENOPTERA

The species of the order Hymenoptera form two more or less distinct groups: a biparental group, in which, under usual conditions, all males and only a few females are uniparental; and a uniparental group, in which, under usual conditions, all females and all males are uniparental.

The sawflies (Tenthredinidae) commonly reproduce uniparentally (Imms, 1934). At least 30 genera of the parasitic Hymenoptera contain one or more species that reproduce uniparentally (Clausen, 1940). In a majority of the 16 species of the phytophagous genus *Harmolita*, studied by Phillips (1920), reproduction is uniparental.

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[1] Paper No. 521, University of California Citrus Experiment Station, Riverside, California.

[2] *Apomixis* is a common name for uniparental procreation by organisms in which the sexual structures are retained (Dobzhansky, 1941).

According to Clausen (1940), it is rather surprising that the uniparental reproduction of females has not yet been found to occur in the Serphoidea.

In some species of Hymenoptera, uniparental and biparental reproduction are racial characters. This is most apparent when such races are segregated geographically, as with those of *Hemiteles areator* and *Eupelmella vesicularis*, which are uniparental in North America and biparental in Europe (Muesebeck and Dohanian, 1927); also with those of *Leucospis gigas*, which are uniparental in Europe and biparental in North Africa (Berland, 1934); of *Trichogramma embryophagum*, which are biparental in North America and uniparental in Europe; and of *Thripoctenus brui*, which are uniparental in Europe and biparental in Japan (Clausen, 1940).

This differentiation in reproduction appears to be dependent on the production of two kinds of eggs: type I, yielding only uniparental females, and type II, yielding biparental females as well as uniparental males. Any species may be capable of producing both type-I and type-II eggs, but most of the eggs deposited by any one species are of one type. This is indicated by the fact that in uniparental species males are relatively uncommon and biparental females extremely rare, while in biparental species males and biparental females usually are common and uniparental females uncommon. In any species the type-I egg and the fertilized type-II egg may be genetically equal.

THE PREVALENCE OF UNIPARENTAL BISEXUALITY

There are relatively few recorded observations on the bisexuality of uniparental broods[3] of biparental species or races. This may have been in part because observers have viewed with suspicion the occurrence of uniparental females in biparental species and have regarded such an occurrence as evidence of faulty technique in the handling of material.

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[3] The term *brood*, as used in this paper, comprises *all* the mature offspring of a single female.

Uniparental females have been recorded as occurring in the biparental species *Apis mellifica* (Mackensen, 1943), *Pteromalus puparum* (Adler, 1894), *Lasius niger* (Reichenbach, 1902), *Lysiphlebus testaceipes* (Hunter, 1910; Webster and Phillips, 1912), *Campsomeris trifasciatus* (Box, 1925), *Ephialtes extensor* (Rosenburg, 1934), *Microbracon hebetor* (Speicher and Speicher, 1938), and in the biparental race of *Diprion polytomum* (Balch, Reeks and Smith, 1941).

Many species hitherto recorded as biparental, because the male sex comprises a large part of the population and mating readily occurs, actually may be uniparental in reproduction. If the females of a bisexual species such as the ichneumonid *Opheltes glaucopterus* produce only unisexual broods (Bischoff, 1927), uniparental reproduction may be suspected. Species are probably biparental if, when reproducing under optimum conditions, the unmated females produce only males.

Apparently, the bisexuality of uniparental populations of Hymenoptera is most marked in the alternate generations of the gall wasps (Cynipidae). The males in the uniparental spring generation are numerous, and mating readily occurs. While mating may or may not be necessary for the genesis of overwintering-generation females, it appears to be a prerequisite of oviposition by the parental spring generation. In certain cynipids and in other species of uniparental Hymenoptera, however, such as *Habrolepis rouxi* Compere and *Encarsia formosa* Gahan, the female appears to have lost the mating instinct.

The bisexuality of uniparental populations varies within species as well as between species. Speicher and Speicher (1938) noted that uniparental females of *Microbracon hebetor* were obtained almost entirely from biparental females resulting from crossing certain strains. The uniparental females regularly constituted about 1 per cent of the total progeny of unmated biparental females. Smith (1941) reported that the uniparental race of *Diprion polytomum* in Canada appeared to consist of strains differing in the frequency of male production.

Apparently, the bisexuality (amphitoky) of uniparental hymenopterous populations verges toward total femaleness (thelyotoky), as in the sawflies, or toward total maleness (arrhenotoky), as in the serphoids.

UNISEXUAL AND BISEXUAL BROODS IN RELATION TO HABITAT AND SEASONAL DEVELOPMENT

Whenever unisexual and bisexual broods have been noted as occurring in a uniparental species, there has appeared to be a distinct correlation between the sexuality of the broods and the type of habitat or the season in which development occurred, the conditions under which one generation developed apparently predetermining the sexuality of the following generation. Such a correlation has not been noted in the case of biparental species, probably because of insufficient data.

Uniparental Species in which Developmental Conditions May Cause the Production of Type-II Eggs

Since the type-II egg is not predominant and usually is rare in most uniparental species developing under optimum conditions, it is in such species that the effect of environmental changes on the production of type-II eggs is most readily discernible.

Male production correlated with temperature. Although Speyer (1926–27) stated that, in the uniparental *Encarsia formosa,* parasitic on the greenhouse whitefly, low temperatures preceded the occurrence of male adults, his data show that the parents of the males developed during a period when temperatures were high, 7 males appearing among 100 specimens that emerged during September.

The uniparental chalcid *Harmolita grandis* oviposits in stems of winter wheat and, like many of the gall-forming cynipids, exhibits generations alternating in habitat, form, and sexuality, a unisexual overwintering generation consisting of perfectly winged females developing from eggs deposited during May, and a bisexual generation in which the females are wingless or imperfectly winged developing from eggs deposited during March (Phillips and Emery, 1919; Phillips, 1920).

Several species of uniparental mymarids of the genera *Anagrus* and *Paranagrus* were reared continuously from January to the middle of September, in Hawaii, where a generation occurred every three weeks. Males appeared only in September, the progeny of females that developed during August, the warmest month of the year in that area (Perkins, 1905).

In the uniparental race of the spruce sawfly, *Diprion polytomum,* males are rarely found in the field, and constitute only about 0.09 to 0.38 per cent of the population. Higher percentages are observed in the warmer regions. When this race is propagated at temperatures exceeding 70° F, the sex ratio may be 1 male to 37 females (Smith, 1941).

The chalcid *Ditropinotus aureoviridis* exhibits a unisexual overwintering generation and a bisexual summer generation (Phillips and Poos, 1921).

The writer has found that, with the uniparental chalcid *Habrolepis rouxi,* the production of unisexual or bisexual broods may depend on the environmental conditions to which the parent females are subjected during their early larval stages. Females that develop at temperatures of 80° F, or less, in healthy *mature* red scale, *Aonidiella aurantii* (Mask.), infesting freshly picked grapefruit are thelyotokous, producing only unisexual (female) broods. Females subjected to a constant temperature of 90° F during early larval development in *immature* hosts are usually either amphitokous or arrhenotokous, producing bisexual broods or unisexual (male) broods.

Male production correlated with nutrition. The uniparental braconid *Microctonus brevicollis,* a parasite of the beetle *Haltica ampelophaga,* in Algeria, exhibits generations alternating in habitat and sexuality, a unisexual generation that develops from eggs deposited in the host larvae alternating with a bisexual overwintering generation that develops from eggs deposited in the adult of the host species (Künckel d'Herculais and Langlois, 1891).

Van Rossum (Bischoff, 1927) observed that the various species of sawflies feeding on alder are to a great extent unisexual, while very closely related species feeding on birch are bisexual.

The chalcid *Prospaltella perniciosi* Tower is bisexual when reproducing on San Jose scale growing on peach trees and unisexual when reproducing on San Jose scale growing on the cow melon (*Citrullus vulgaris*) in the laboratory (Flanders, 1944).

The bisexuality of the uniparental gall-forming eurytomid *Trichilogaster acaciae-longifoliae* varies with different species of host plants, *Acacia longifolia* var. *floribunda* yielding males and females in about equal numbers, while the number of males yielded by *A. longifolia* is about 1 per cent of the population. It is significant that in ovipositing on *A. longifolia,* the female selects only the developing flower buds, whereas on *floribunda* it oviposits in the tips of young branches as well as at the points where flower buds develop (Noble, 1940).

Biparental Species in which Developmental Conditions May Cause the Production of Type-I Eggs

Species in this group are entirely or largely biparental in reproduction; that is, most of the females are arrhenotokous, and the few that produce type-I eggs are amphitokous. In this group it should be noted that the sex ratio may be modified by post-oogenic factors.[4]

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[4] In species that reproduce biparentally, the sex ratio of any generation may be changed by the differential mortality of eggs and larvae. When fertilization is necessary for the production of females, then the sex of the deposited egg is determined by the rate of oviposition and the degree of preferential oviposition (Flanders, 1939).

In 1876, Adler (1894) observed uniparental bisexual production in the biparental chalcid *Pteromalus puparum*. The broods of two unmated females consisted of 75 males and 5 females, and of 45 males and 4 females, respectively.

In a series of experiments by Kelly and Urbahns, in 1908, (Webster and Phillips, 1912), 3 unmated females of the biparental braconid *Lysiphlebus testaceipes* produced broods as follows: 70 males and 3 females, 101 males and 6 females, and 67 males and 1 female. An unmated female of one of these broods reproduced so that a second generation of uniparental females was obtained.

Hunter (1910) records bisexual broods from 7 unmated females of *Lysiphlebus testaceipes*. Hase (1922), from an unmated female of *Microbracon hebetor,* reared 16 males and 14 females. Moursi (1943) obtained 3 uniparental females of *Mormoniella vitripennis* in a brood consisting of 162 individuals. The time of their emergence indicated that they were derived from the first eggs deposited by the mother. These were the only females among 15,925 individuals reared during the experimental work.

The wheat stem sawfly, *Cephus cinctus,* has been assumed to be biparental in most of the infested districts of Canada, since in these areas the sexes occur in about equal numbers. Significantly, however, in certain districts of Alberta, where the sawfly recently has become established, only females are produced (Farstad, 1938).

Mackensen (1943) states that in the domestic strains of the honeybee in the United States, uniparental females occur more commonly than hitherto believed. Of 50 virgin queens producing enough offspring to make a good test, 21 produced bisexual broods. The number of female offspring produced by any one virgin queen was estimated not to exceed 1 per cent.

In certain species of solitary bees of the genus *Halictus*, Fabre (1879) observed uniparental bisexual generations that developed during the summer, alternating with biparental unisexual generations that developed during the spring. In this case the females of the unisexual generations produced type-I eggs *in addition to* type-II eggs. This is the reverse of the condition noted in the case of *Harmolita.*

The Production of Type-I and Type-II Eggs in the Cynipids

The cynipids present many interesting examples of a definite correlation between alternating habitats and seasons and alternating bisexual and "agamic" (unisexual) generations. In certain species the females of the "agamic" (supposedly biparental) generation are of two types (Patterson, 1928b): the ovaries of one type of "agamic" female are thelyotokous, and those of the other type arrhenotokous. The ovaries of the females of the bisexual generation are largely if not entirely of one type, however, as indicated by the fact that their progeny are usually female.

The occasional unfertilized type-II egg deposited by the bisexual generation may not hatch because of the unsuitability of the plant tissues in which it is deposited. This is not a unique phenomenon, for the unfertilized type-II eggs deposited by *mated* females of several species of the chalcid genus *Coccophagus* never hatch (Flanders, 1937). The few males of the "agamic" generation reaching maturity may never mate, since their sibs lack the mating instinct (Patterson, 1928a).

The alternation of a generation in which females are of one reproductive type with a generation in which the females are of two reproductive types appears to be an effect of nutrition—an effect possibly enhanced by differences in temperature during development. The chemical constitution of the concentrated nutriment in which the larvae of each alternating generation are immersed may differ because of differences in the reaction of different parts of the host plant to the secretions of the larvae.[5]

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[5] The form of the gall produced by the cynipid depends on the species of insect rather than on the species of plant (Kinsey, 1929). The host plant produces the gall under stimulation of a glandular fluid secreted by the cynipid larva (Triggerson, 1914).

Adler (1894) suggested that any differences in cynipid larvae result only from differences in the duration of their development. Duration of development, however, may be an effect of nutrition, as has been observed in the honeybee. It is interesting to note that male production by uniparental females of *Cladocera* (Crustacea) is promoted by environmental factors that lower the rate of metabolism (Banta, 1937).

If, in the bisexual generation of cynipids, fertilization is not necessary for reproduction, then the cynipids follow the apparent rule, that the production of the type-II egg is correlated with high temperature during development. In the bisexual generation—that is, the generation in which males are numerous and females are of one type—the females are exposed to comparatively low temperatures during early larval development and theoretically should be thelyotokous. The "agamic" generation, consisting of females of two types (thelyotokous and arrhenotokous), is exposed to comparatively high temperatures during early larval development.

Since it is probable that it is the influence of the environment on the immature stages of the "agamic" generation which determines the proportion of type-I and type-II eggs produced by that generation, it follows that, if the broods produced by the females of the "agamic" generation are either all male or all female, then the parent of each brood developed under one of two sets of conditions, as regards food and, possibly, temperature. If, as in the case of *Neuroterus niger,* a cynipid female tends to deposit all its eggs within a very limited area, as, for example, in one oak leaf (Patterson, 1928b), all the "grandchildren" of a bisexual-generation female may be entirely female or entirely male (Doncaster, 1917), since their parents were reared together under uniform conditions..

The females of the alternate generations of cynipid gall wasps are so different morphologically that they have been described as distinct species. This difference is ascribed to nutrition (Salt, 1937). The fact that only a portion of the females of the "agamic" generation are thelyotokous indicates that ovarian dimorphism in this generation may result from differences in exposure to either nutritional or atmospheric conditions during development.

In the case of the uniparental cynipid *Rhodites rosae*, in which there is no alternation of generations, males and females are produced in a ratio of 1 to 130. A few galls yield only males; others yield both sexes (Callan, 1940).

It is interesting to note that the eggs produced by cynipid inquilines may be only of type II, although the females may have developed in host galls formed by host larvae destined by environmental factors to produce eggs of type I (McCracken and Egbert, 1922).

A MECHANISM PRODUCING EGG TYPES I AND II, SUBJECT TO ENVIRONMENTAL CONTROL

In uniparental species of Hymenoptera, the mechanisms for retaining the diploid number of chromosomes vary widely (Speicher, 1937). In the uniparental species *Habrolepis rouxi*, it has been observed by the writer that, at normal temperatures, bisexual broods usually are produced by only a few females, and that males form only a very small part of broods developing under optimum conditions. At temperatures of about 80° F, or less, a female that happens to have the capacity for producing type-II eggs always begins to deposit such eggs within about a week after emergence from the host. Since the egg-production period of a female is considerably longer than the developmental period of its offspring, and the sexuality of the brood is apparent soon after the offspring begin to emerge as adults, it is possible to segregate those females that lack the capacity for male production *before* they deposit all their eggs. This would be an improbable situation if uniparental females were derived from type-II eggs as a result of some modification of the meiotic process.

Schrader (1920) suggested that uniparental reproduction in the cynipid *Rhodites rosae* might be an effect of the multiplication of chromosomes in the oogonia, so that after reduction the nucleus of the egg is diploid. The work of Speicher and Speicher (1938) on the biparental *Microbracon hebetor* indicates that type-I eggs in the higher Hymenoptera may be formed in this way. The occasional uniparental female of *M. hebetor* developed from tetraploid eggs in which meiosis was normal, and which probably originated in patches of tetraploid tissue in the ovary.

Since tetraploidy is usually accompanied by a reduction in number of cells, a relatively low ovipositing capacity would be expected in females producing type-I eggs. The uniparental race of *Diprion polytomum* has a much lower reproductive capacity than has the biparental race (Smith, 1941). Smith, however, was unable to distinguish any polyploid nuclei in the germinal cells of *D. polytomum,* although he found them to be numerous in the somatic tissues.

According to Smith (1941), the amount of chromatin material in the oogonia of the uniparental race of *Diprion polytomum* exceeds that of the biparental race by only 2 chromosomes, there being 14 chromosomes in the uniparental race and 12 in the biparental race. In both races meiosis is apparently normal, but in the uniparental race Smith considered chromosome reduction to be compensated for by refusion of the second polar body with the female pronucleus.

On the other hand, the type-I eggs in the uniparental sawfly *Thrinax macula* are said to remain diploid by undergoing one maturation division (Peacock, 1938).

Tetraploidy of the oogonia, however, seems to be the simplest of the methods by which type-I eggs may be produced, and the one most likely to be brought about by environmental conditions.

It may be significant, from the standpoint of the doubling of the chromosomes in the oogonia, that, with the cynipid *Dryophanta erinacei,* Triggerson (1914) noted a larger larval abdomen in the generation destined to produce type-I eggs than in the generation destined to produce type-II eggs. A similar phenomenon occurs in the case of *Diprion polytomum,* the uniparental race of which has a higher proportion of large individuals than the more rapidly developing biparental race. In insects, the amount of food consumed during the larval period may determine, within specific limits, the size of the mature individual. However, in the two species cited, the morphological differences between the uniparental and biparental forms, probably induced by the *quality* of the food rather than by the amount, are instances of dimorphism.

Brues (1928) suggests that with *Pelecinus polyturator,* the female of the supposedly uniparental race inhabiting temperate North America is tetraploid, since it is so much larger than the female of the supposedly biparental race inhabiting tropical America.

An ovarian dimorphism in the Hymenoptera is indicated by the occurrence of egg types I and II. An ovary is either arrhenotokous (all eggs diploid), thelyotokous (all eggs tetraploid), or amphitokous (eggs tetraploid and diploid). When the uniparental offspring of a female consist of equal numbers of males and females, it is quite possible that one ovary is thelyotokous and the other arrhenotokous.

INFLUENCE OF ENVIRONMENT IN DETERMINING TYPE OF OVARY

Observations on the sexuality of the broods of *Habrolepis rouxi,* when parasitic in red scale (an unnatural host), indicate that one type of ovary may be converted into another type if the developing larva is subjected for a period to certain nutritional conditions which are not optimum for reproduction.

Qualitative differences in nutrition are known to be the cause of dimorphism in certain Hymenoptera (Salt, 1937; Schmieder, 1933). It is significant that the hymenopterous ovary is a plastic organ which reacts to trophic stimuli (Wheeler, 1928). The number of ovarioles in the adult may be determined by the nutrition of its early larval stages. It is well known that, in the social Hymenoptera, morphological and physiological differences exhibited by the ovaries of workers and queens result from differences in larval nutrition. Such differences may initiate differences in rates of development and thus have a dimorphic effect (Power and Melampy, 1943). When the worker appropriates for itself food that ordinarily would be fed to larvae selected to become queens, its rudimentary ovaries undergo development and it becomes capable of depositing unfertilized eggs. Whether such eggs develop into males or into females, as in the Cape honeybee (Jack, 1917), also may depend on nutrition.

Experiments with Habrolepis rouxi. This is an endoparasitic uniparental species whose development is readily affected by the kind of plant, or by the condition of the plant, fed upon by its host (Flanders, 1942b). During the several years when mass production of *H. rouxi* was being carried on in the insectary at the University of California Citrus Experiment Station, it was noted that the sexuality of the parasite populations seemed to be affected by the type of host plant used. Reproduction on squash fruits resulted in a higher proportion of males than reproduction on lemon fruits (Flanders, 1942a). A similar difference was noted in parasite populations reared from red scale on freshly picked grapefruit and from red scale on old grapefruit—that is, grapefruit held for 30 days at 80° F. Significantly, the viability of red scale is noticeably less on old grapefruit than on fresh grapefruit.

The sexuality of populations of *Habrolepis rouxi* also seemed to be affected by the maturity of the insect host, a higher proportion of males occurring when the red-scale host was immature than when it was mature. A complicating factor was a tendency toward male production seemingly correlated with high temperatures, males being relatively more numerous in summer than in winter. A series of experiments was therefore undertaken to determine the relative effects of host plant, maturity of insect host, and environmental temperature on the occurrence of males.[6]

Red scales between 35 and 55 days old, when grown at a constant temperature of about 80° F, are most suitable for the development of *Habrolepis rouxi.* Scales less than 20 days old are least suitable, for in such scales development of the parasite is considerably prolonged. Suitability increases with the age of the scale, up to about 35 days, and is probably an effect of chemical differences.

In preliminary tests using groups of female parasites, 24 females reared in red scale over 35 days old and *equal in maturity* were all thelyotokous, for they had 667 progeny, all of which were female. The progeny of 16 females reared in young scale (16 to 27 days old when attacked) consisted of 172 females and 9 males, while the progeny of 16 females reared from nearly mature scale (26 to 36 days old when attacked) consisted of 243 females and 5 males. The progeny of each group were reared in scales of equal age, on lemons, at 80° F.

The data in Table I, based on the broods of 91 isolated females, indicate that the temperature during the development of the female accentuates the effect of nutrition on the sexuality of its brood. In most cases the broods were incomplete because the parents were allowed to oviposit for only about 20 days. At 80° F, the life of the ovipositing adult may be as long as 40 days. However, as noted previously, the sexuality of a brood is apparent soon after emergence begins.

As Wigglesworth (1939) has stated, growth, or reproduction, or some other function, may be adversely affected toward the limits of the temperature range of the species. In the case of *Habrolepis rouxi,* if the entire larval growth takes place at 90° F, pupation is inhibited unless the temperature is lowered. But pupation at 90° F is not inhibited if exposure to this temperature does not occur until after larval feeding ceases. Under optimum conditions for maximum reproduction, all females are thelyotokous. Females that develop in immature hosts on fresh grapefruit, or in mature hosts on old grapefruit, may be amphitokous. In either case, however, subjection to high temperature is necessary to cause these females to become arrhenotokous.

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[6] The experimental data obtained in the reproduction of *Habrolepis rouxi* were collected, under the writer's supervision, by Glenn L. Finney, superintendent of the insectary, whose assistance greatly facilitated this study.

TABLE I

TYPES OF OVARIES, AS DETERMINED BY THE SEXUALITY OF THE BROODS, OF 91 FEMALES OF HABROLEPIS ROUXI, CORRELATED WITH CERTAIN ENVIRONMENTAL CONDITIONS TO WHICH THE FEMALE PARASITES WERE SUBJECTED DURING THE EARLY STAGES OF THEIR DEVELOPMENT

Test	Age (in days) of red scale* when exposed to female parasites	Host plant	Temperature (degrees Fahrenheit) during developmental stages of female parasites	Females grouped according to ovarian types†		
				Thelyotokous	Amphitokous‡	Arrhenotokous
A	17+	Squash	80°	14	1	
B	27±	Fresh grapefruit	68°–73°	8	2	
C	28	“ “	80° (larval stage, 90°)	2	2	
D	27	“ “	80° (early embryonic stage through larval stage, 90°)		8	
E	27	“ “	80° (early embryonic stage through first half of larval stage, 90°)	3	6	1§
F	34+	“ “	80°	6	1	
G	39	Old grapefruit¶	70°–73°	8		
H	39	“ “	80°	9		
I	39	“ “	80° (embryonic stage, 90°)	8	2	
J	39	“ “	80° (early embryonic stage through first half of larval stage, 90°)	1	6	3

* Red scale in this experiment were mature when about 38 days old.

† The offspring of the 91 female parasites (2,548 females and 384 males) were reared in red scale on grapefruit, at a constant temperature of about 80° F. The type of ovary apparently had no influence on the average number of offspring per female.

‡ The ratio of males to females from amphitokous ovaries ranged from 1 : 46 to 21 : 26.

§ This female had 71 male offspring.

¶ By "Old grapefruit" is meant fruits held for 30 days at 80° F before infestation with red scale.

Developmental temperatures alone do not determine the bisexuality of uniparental populations. Numerous tests with the biparental species *Mormoniella vitripennis* by Moursi (1943), and with *Microbracon hebetor* by the writer, using low temperatures, have failed to yield uniparental females. The writer also subjected numerous individuals of the uniparental species *Nemeritis canescens* Grav. to high temperatures during development without bringing about the production of males.

Prebble (1941) has called attention to the fact that the unisexuality of the uniparental race of *Diprion polytomum* is correlated with the occurrence of diapause, and that bisexuality is correlated with the lack of diapause. This correlation indicates that hormone activity may be the nutritional factor involved in the dimorphism of the ovaries. "Diapause" generations may alternate with "nondiapause" generations, the diapause being determined long before it becomes apparent. It is of interest to note in this connection that silkworm eggs incubated at 25° C (77° F) tend to produce moths that deposit "diapause" eggs, while those incubated below 15° C (59° F) tend to produce moths that deposit "nondiapause" eggs (Wigglesworth, 1939).

BIPARENTAL AND UNIPARENTAL REPRODUCTION AS A FACULTATIVE PHENOMENON

In the biparental reproduction of females and the uniparental production of males, Dobzhansky (1941) points out (1) that there may be freedom to form gene combinations although the supply of hereditary variations is limited, and (2) that functional haploid males provide a means for the rapid elimination of unfavorable mutant genes, if the genes that are recessive in females have similar phenotypic effects in both sexes.

A species in which the reproduction of females is entirely uniparental may be in a phylogenetic "blind alley." Peacock (1925) points out that in the Tenthredinidae (sawflies), a group in which uniparental reproduction is of long standing, there is a stereotypy of form. It is not unlikely, however, that a uniparental species subjected to a change in environmental resistance may produce a biparental race, provided the mating instinct is activated. Conversely, a biparental species subjected to certain environmental conditions may produce a uniparental race.

The fact that Kelly and Urbahns (Webster and Phillips, 1912) obtained from the biparental species *Lysiphlebus testaceipes* a line of uniparental females which was maintained for two generations indicates that such reproduction is facultative. Kelly and Urbahns suggested that such uniparental reproduction might be an adaptation for carrying the parasite through periods of host scarcity.

No observations have as yet been made on the occurrence of biparental females in a uniparental species, since recognition of such females depends on the occurrence of a difference in form or in chromosome number.

The adults of the uniparental and biparental races of *Diprion polytomum* differ morphologically and in chromosome number. Since the females of the uniparental race have exhibited the mating instinct, there is a possibility that a biparental line may be produced from the uniparental race. A uniparental female with the morphological characteristics of the uniparental race was produced by the biparental race (Smith, 1941). When this occurred, it was assumed that there had been an accidental mixture of breeding stock. It is possible, however, that this female was derived from a type-I egg.

According to Whiting (1943), biparental reproduction with haploid males must have been attained very early in the evolutionary history of the Hymenoptera. Uniparental reproduction in this order would therefore be secondary. The fact, however, that uniparental reproduction appears to be relatively more common in the primitive Hymenoptera than in the higher forms, such as the serphoids, indicates that uniparental reproduction in the Hymenoptera was derived directly from biparental reproduction in which the males were diploid, and not from biparental reproduction in which the males were haploid.

SUMMARY

In the Hymenoptera, the uniparental production of fe-

males is a fairly common phenomenon. All normal males appear to be uniparental.

The species of this order fall into two groups: (1) those species which generally reproduce biparentally, commonly producing uniparental males and occasionally producing uniparental females; and (2) those species which reproduce uniparentally, but with only the occasional production of males, although certain females of this group may produce only male progeny. The occurrence of the different types of reproduction appears to be dependent on a dimorphic condition of the ovaries—that is, ovaries that are either thelyotokous or arrhenotokous.

The life histories of certain species of uniparental Hymenoptera recorded in the literature, and data from observations on the reproduction of the red-scale parasite *Habrolepis rouxi*, indicate that the gonads of the immature female larva, which are potentially thelyotokous, may develop into ovaries that are partly or completely arrhenotokous. This change may result from a change in the nutrition of the larva, accompanied by a change in environmental temperature. It is significant that, in the species noted, the thelyotokous condition is associated with relatively low temperatures, and the arrhenotokous condition with high temperatures.

Observations on *Habrolepis rouxi* indicate that thelyotoky in the Hymenoptera does not necessarily involve a modification of the meiotic process. Thelyotoky commonly may result from a doubling of the chromosomes in the primary oogonia.

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THE RELATION BETWEEN HEREDITY, SEXUAL ACTIVITY AND TRAINING TO DOMINANCE-SUBORDINATION IN GAME COCKS[1, 2]

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ALLEE (1942) and Allee and Collias (1940) have studied environmental and hormonal factors in relation to dominance-subordination patterns in vertebrates, and Murchison (1935a, 1935b, 1935c) and Guhl (1942), who also studied domination-subordination, used the discrimination cage for investigating social discriminations in domestic varieties of chickens. In the experiments to be described, especial attention was given to factors which are of significance in the development of dominance in game cocks.[3] The scarcity of knowledge in regard to development of dominance in this interesting and least understood breed of chickens makes additional information about the behavior pattern highly desirable.

MATERIAL AND METHODS

Game cocks used for experimentation were obtained by crossing Allen Roundhead hens with an Arkansas Traveller cock, and the hens obtained were back-crossed with the Arkansas Traveller. Some of the first-generation cocks were used, but their behavior was found to be essentially the same as that of the second generation cocks. In some experiments, behavior in first- and second-generation cocks was checked against behavior in adult Dominique, White and Allen Roundhead game cocks. Hybrid Brown Leghorn, Rhode Island Red, Brown Leg-

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[1] All observations were made at Decatur, Alabama, prior to 1923.

[2] The author is indebted to Dr. A. M. Moll, Grand Rapids, Mich., and Mr. J. A. Davidson, Poultry Department, Michigan State College, for reading the manuscript and for valuable criticisms.

[3] Moll states (personal communication) that "true gameness is a recessive characteristic." He adds "some cross-bred cocks (cocks obtained by crossing games with other breeds of chickens) fight fiercely, and are apt to be mean, *i.e.,* they will attack man and other animals."

horn and Barred Plymouth Rock cocks were used in other experiments.

All game chickens used in the experiments were bred and hatched in an enclosure which is described in Fig. 1 as a breeding pen. It consisted of a small house and an attached yard, about 16 feet wide and 20 feet long, fenced with poultry wire about 60 inches high. The wings of all game chickens were clipped before the chickens were left in the breeding pen. Other fences described in Fig. 1 as hogwire and board fences were constructed from 32-inch heavy fenestrated wire and 6-inch boards. In this type of fence, the lower surface of the wire was in contact with the ground and the upper surface was 6 inches below the lower member of two parallel boards that were spaced

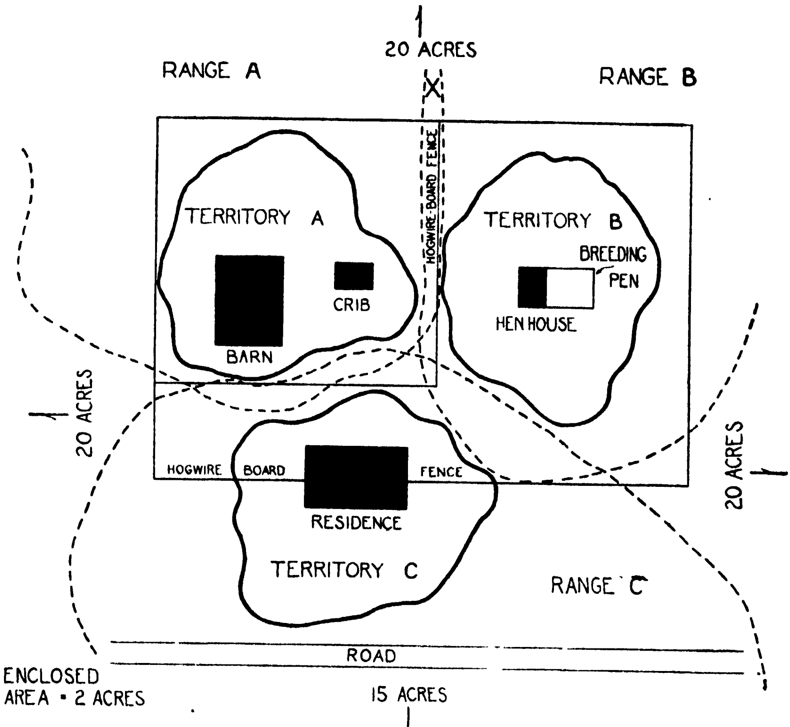

FIG. 1. A diagram showing the relations between territories and ranges. The term, "walk," as used in the text, refers to a territory and the range adjacent to it.

about 6 inches apart. All chickens other than the games were bred and reared on the walks shown in Fig. 1.

In all experiments in which trained cocks were used, the cocks were trained on top of a table (4 feet wide, 6 feet long and 4 feet high), which was covered by a layer of cotton one-fourth inch thick. The following method was used in training: A cock was put on top of the table, and the trainer's hands were placed over or beneath the cock's wings. The cock was exercised by pushing the bird to the left and then to the right for 5 to 10 minutes. Other muscles were exercised by pushing the cock backward and forward for an additional 5 to 10 minutes. Wing muscles were strengthened by releasing the cock 2 feet above the table top; this procedure was repeated for 5 to 10 minutes. Birds in training were kept in small individual pens, and care was taken to keep a cock away from other cocks until the training was completed. The length of the training period depends on the physiological condition of the cock at the beginning of the training period, but with most cocks 2 to 3 weeks was usually adequate. A cock in good physical condition had a well-developed muscular system and showed little fatigue at the conclusion of a training period.

Fig. 1 shows that the breeding yard was divided into territories and ranges. A territory and the range adjacent to it will be referred to in this paper as a walk. Each of the walks, which was surrounded by a supplemental range of 15 or more acres, was occupied by a game cock and 15 to 20 hybrid hens.

OBSERVATIONS

The Relation Between Heredity and Dominance. The relation between heredity and dominance was studied by matching dominant game cocks with dominant hybrid cocks (Rhode Island Red, Brown Leghorn and Barred Plymouth Rock Hybrids), and carefully noting the behavior of each while the matches were in progress. One example will be used to illustrate the importance of heredity in dominance-subordination. Walk C had been dominated by a large hybrid Brown Leghorn cock for several years. This bird was matched on walk C with a dominant untrained game cock by releasing the game cock at a distance of about 30 feet from the Leghorn hybrid. Both cocks crowed, and the game moved in and hit the Leghorn with both spurs. After about 10 minutes in which the action was fast, it was seen that the Leghorn was tiring. The game continued to carry the attack to the Leghorn, and the Leghorn fought back defensively, but during the match the Leghorn gave way to the game. In one of the encounters made late in the match, in which each cock was the recipient of several blows, the spur of the game broke the skin beneath the eye of the Leghorn, and then the Leghorn discontinued the fight and ran. Both the Leghorn and the game were left on the walk and encounters between the two cocks were frequently observed, but the matches were invariably terminated by the Leghorn. In this match and in other matches between games and domestic varieties of cocks, it was observed that the games tolerated more punishment, and that they were shiftier, faster and less clumsy than domestic cocks. Such marked differences in behavior were doubtless dependent on the hereditary background of the cocks.

Some observations suggested that methods of attack used by some varieties of game cocks may be hereditary. Kentucky Dominique cocks, in most matches, were above opponents before striking, and as a consequence, the Dominiques did more flying in matches than the Allen Roundhead. Roundheads moved to within striking distance and then struck forward or toward either side of opponents. When the Roundhead directed the attack toward the right or left side of an opponent, the legs and feet were brought forward in a sweeping motion so that spur points hit their opponent's body. If opposing cocks eluded their Roundhead opponents by flying upward, the Roundheads were sometimes successful in changing the direction of blows, so that the tips of their spurs hit the ventral body surface of opposing cocks. At the completion of such maneuvers, the Roundheads were frequently lying on their backs, with both legs extended upward. The high, flying type of attack, as used by the Dominiques and the shifty, ground attack, used by the Roundheads, appear to be independent of training.

The Relation Between Isolation, Sexual Activity and Dominance. Game cocks were subordinated by raising game chicks from hatching time to sexual maturity on walks with dominant game cocks. A cock subordinated in this way, when approached by the dominant cock, would run and remain at a distance that made escape possible without engaging the dominant cock. While making these observations, the author noted that subordinated cocks tread fewer hens than dominant ones. Such a behavior pattern in subordinated birds may be due to a decreased sexual activity or to the hens' preference for dominant males, although Guhl (1942) considering the same question states (p. 137): "The fact that the social discrimination of males by females is significant does not necessarily imply that the hens showed a 'preference' for 1 as the dominant male, as their reactions may have been negative discriminations of 11 and 111 and positive only to 1."

Some observations suggest that isolation and sexual activity are important factors in the development of dominance in game cocks. Only one experiment will be described which is in support of this view, although the observations were repeated on many subordinated cocks. An 11-month-old game cock, subordinated to a cock on walk A, was removed from the walk, and left on a nearby farm with 15 or more hens for 3 months. Occasional observations were made on this individual, and it was found that the behavior pattern changed from that of a subordinated bird to that of a more dominant one.

This change in behavior pattern was characterized by an increase in frequency of crowing, while increased sexual activity was suggested by the increase in the number of hens trod. When the young cock was first left at the farm, it appeared as though he were trying to make himself as inconspicuous as possible, but after 4 weeks on the farm, he had straightened and his posture resembled that of a dominant bird. Two months after these observations were made, the young cock was returned, to ascertain whether increased sexual activity and isolation with a strange flock of hens had developed superficial[4] or real dominance. To make a reliable test, the young cock was matched with the cock on walk A to which he had been subordinated 11 months previously. On sight of the cock on walk A, he vigorously attacked the older cock and continued the attack until both were exhausted. These observations and other observations made on subordinated cocks suggest that sexual activity[5] and isolation are important factors in the development of dominance in subordinated birds.

Territories and Ranges of Game Cocks. Allee (1942) states (p. 143): "All group organizations among birds are apparently based on the ability of birds to recognize and remember their flock-mates as individuals. When territory enters as a factor, recognition of the individual's territory also becomes a part of the group reaction system."

To obtain a more accurate understanding of recognition of territory and of individuals by game cocks, it was found expedient to divide walks shown in Fig. 1 into territories and ranges. Each division is used in the same sense as it was used for small mammals by Burt (1940). He defines a territory as an area around a home-site, defended against members of the same species, while a home range is an area adjacent to a territory not necessarily defended, which is used for foraging.

Several years later (1943, p. 351) this author states: "Home range then is the area, usually around a home-site, over which the animal normally travels in search of food. Territory is the protected part of the home range, be it the entire home range or only the nest. Every kind of mammal may be said to have a home range, stationary or shifting. Only those that protect some part of the home range, by fighting or aggressive gestures, from others of their kind, during some phase of their lives, may be said to have territories.''

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[4] An individual exhibiting superficial dominance when matched with a true dominant will discontinue the match after a few minutes of fighting.

[5] According to Moll, sexual activity is not necessary for the development of dominance. Isolation is just as effective as isolation and sexual activity.

It was found during the course of these observations that cocks usually remained within boundaries of their respective walks. On occasions, cocks would enter ranges of adjacent walks, *i.e.*, at feeding time or while foraging. Territories, however, were always respected. This is well illustrated by the following observations: Cock A and hens frequently foraged on range A at a distance of 100 yards from territory A and at such times cocks B and C did not trespass on territory A. Under these conditions, recognition of territory by B and C may be similar to the mammalian conditioned reflex. If this is the case, both the individual and the individual's territory were concerned with territorial recognition. Moll states (personal communication) that territorial recognition was abolished in a game cock when hens were removed from his walk. When this was done the cock fought it out with the cock in an adjacent walk for domination of the flock in the adjacent walk. This would indicate that the presence of hens on a walk was a limiting factor in territorial recognition.

When cocks were foraging on ranges, some distance from their respective territories, recognition was made of the individual. This was well illustrated by the following observation: It was noted that cock B and hens frequently foraged at X (Fig. 1), an area frequented by cocks B and A. When X was occupied by B and hens, and A and hens moved toward X, the presence of both cocks was soon established by crowing. Cock B and hens continued to forage, although the foraging course was altered by B moving to the right into range B. Likewise, cock A and hens continued to forage, but moved to the left into range A. Neither cock made any pretense of defending the range at X.

Fights between dominant cocks rarely occurred, as walks were large enough to decrease the frequency of unavoidable meetings of cocks while foraging. Sexual competition between cocks was at a minimum as each walk was supplied with 15 or more hens. Cocks B and C occupied their respective walks for over 12 months, and in this period fights which were dependent on the individual initiative of one or both cocks were never observed. Thus, the contention of Noble (1939) "that no two hens, or roosters can remain very long together in the barnyard without establishing which is superior to the other" is acceptable only when applied to a barnyard of a limited area.

The Relation Between Dominance and Training. In a study of the relation of behavior to training, cocks were trained as described in the preceding pages. A cock, when returned after training to the walk which he had occupied prior to training, usually established his presence by crowing and beating the wings against the body. If the trained cock observed another cock on an adjacent walk, he usually moved into the adjacent walk to attack. A trained cock, when taken into the territory of another cock and then released, would aggressively attack the dominant cock in the territory. Action in matches made under either of these conditions was fast, and the trained bird continued to carry the attack to the opponent. As the match progressed, the effects of training were evident. The trained cock was shiftier, showed less fatigue, and also gave the impression of being a superior fighter. In matches made between trained and untrained game cocks, care was taken to match cocks of about the same age and weight, so that differences in behavior observed while the match was in progress could be more closely correlated with training. The observations described in the preceding paragraph suggest that training was an important factor in developing aggressiveness and stamina in game cocks, and that increased aggressiveness and stamina, under the conditions described, may be closely correlated with dominance.

DISCUSSION

Scott (1942) finds that in sage grouse, the social organization on mating spots consists of a central group of hens, a master cock which dominates the group, a sub-cock or chief rival cock and several guard cocks. Master cocks are aggressive, vigorous and usually larger than other cocks in the organization. This author also found that master cocks always mated a greater number of times than sub-cocks or guard cocks. If it is assumed that increased frequency of mating in sage grouse is correlated with increased sexual activity, the development of dominance in sage grouse cocks and game cocks is fundamentally the same. It was suggested in the preceding pages that training increases aggressiveness and stamina in game cocks, and that both aggressiveness and stamina seem to be important factors for the development of dominance in grouse. A further comparison of dominance in game cocks and sage grouse cocks shows that the behavior of sage grouse guard cocks and young subordinated game cocks is similar in several respects. Both recognize and accept the dominance of other cocks, and in this acceptance guard cocks remain outside the breeding spots during the mating season; consequently, such cocks mate only occasionally. Likewise, subordinated game cocks remain in the outskirts of a walk, some distance from the flock, and mate when the vigilance of the dominant cock slackens. Master cocks dominate grouse social organizations by threatening and fighting, and domination of a walk by a game cock is maintained by essentially the same methods.

It is evident that a comparison of social organization and dominance in grouse and game chickens should be used with considerable reservation as this method, if carried to the extreme, may yield false conclusions. This comparison in no way constitutes proof that social organization is essentially the same in both sage grouse and game chickens, and it is offered with the idea that such comparisons may give clues to investigations which may further elucidate the biological and environmental factors that determine the avian behavior pattern.

SUMMARY AND CONCLUSIONS

Observations made on game chickens, on walks with an area of one or more acres, suggest that dominance-subordination and social organizations are determined by factors which on final analysis are closely related to the hereditary background of the individual. Courage, aggressiveness and shiftiness, all of which are important in domination-subordination, are hereditary, as these characteristics are exhibited to a greater degree in games than in the common domestic fowls considered. Other factors, such as training, isolation and sexual activity, aid in the expression of these latent hereditary characteristics. The results also suggest that identical environmental factors exert less effect in directing the behavior pattern toward dominance in other breeds of chickens considered than in game chickens.

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"NICKING" IN RELATION TO SEXUAL MATURITY OF S. C. W. LEGHORNS[1]

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THE concept of nicking is widely accepted among poultry and animal breeders. In general the term "nicking" is used in reference to matings which are particularly successful by the quality of offspring produced. More specifically, if the progeny of a pair of given parents is superior to that expected by the breeder on the basis of his estimates of the genotypes[2] of the sire and dam, usually from their phenotypes, the mating is said to have nicked well. The consequences of the belief in the existence of this phenomenon have not been particularly emphasized in the past. Yet its implications are rather far-reaching, since, should nicking be of frequent occurrence, it would mean that the results of a progeny test must be taken to refer to the particular combination of parents rather than to the sire and dam individually. In such a case the death or incapacitation of one of the mates (particularly of the sire) would demote the other from the status of a proven breeding bird and require its retesting with a new mate. On the other hand, should nicking be non-existent or unimportant, once a bird has had a successful progeny test with one mate, its genotypic worth may be considered established.

From the genetic point of view the term "nicking" may be considered to be due to two different phenomena: (1) gene interaction of the complementary, epistatic or generally non-additive type, and (2) character interaction. In the final analysis such distinction is undoubtedly arbitrary, but from the standpoint of actual breeding practise it is of importance to consider the two types separately. The first is probably the one more familiarly used in reference to characters not broken down into component factors. The second, referred to by Richey (1942) as "mock-dominance," is well illustrated by the inbred tomato lines cited by Powers (1944), where a cross between one line with a small number of fruits of large weight and another line characterized by many small fruits produced a greater total yield (number of fruit × average weight of fruit) than either parent.

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[1] It is a pleasure to acknowledge the generous advice of Dr. Jay L. Lush, of Iowa State College, in the interpretation of the data presented here.

[2] The terms genotype and genotypic are used here, unless otherwise specified, to designate all hereditary effects including non-additive ones.

An analogous situation may be imagined with respect to egg production in chickens, which can be considered as the expression of interaction of several independently determined characters (Lerner and Taylor, 1943). Thus should a late maturing line with a high rate of production be crossed with an early maturing but low-rate line, it would be reasonable to expect the total number of eggs laid by the progeny of such a cross to exceed the records of the parents. This situation in a certain sense is not nicking at all, since additive gene action alone could lead to it, and since the superior results from the cross would be predictable by the breeder in possession of information on the component characters as well as on the total yield of the parental lines.

The first type, that of gene interaction, may in some cases resolve itself into character interaction, should a breakdown into component factors be made. However, in many cases the breeder may be dealing with characters which can not be broken down any farther either because they are the direct result of gene action or (which is certainly more likely the case with economic characters) because of our incomplete knowledge of the physiology of their development. Some of the components of egg production (Lerner and Taylor, *loc. cit.*) belong to this category, and may then be examined for the existence of the nicking phenomenon.

The amount of data necessary to distinguish nicking from sampling deviations is considerable, and special experimental designs would be highly desirable to achieve this purpose. Some information, however, may be obtained from records already available. Thus it was found that the necessary data were at hand for the character of sexual maturity, since sufficiently large numbers of offspring from each mating were measured for this character before mortality reduced them to numbers too low for the intended purpose.

DESIGN OF ANALYSIS

In the mating records of the University of California flock of Single Comb White Leghorns a series of 31 diallel sets of matings (that is to say, where two dams have been mated at different times to each of two males), producing an adequate number of female offspring, has been located. In five of these sets the mated group contained three dams, and in one case four dams. Thus the total number of sires involved was 62 (31 × 2), while the number of dams included in the study was 69 $(25 \times 2 + 5 \times 3 + 1 \times 4)$. Discounting duplication of the same individuals in different sets, the number of sires was 49 and that of dams 64. In each set the number of pullets, for which the age at first egg (the usual criterion of sexual maturity) was available in the smallest full-sister family, determined the number of pullets selected for study. Thus in a set where the four full-sister families had respectively 3, 5, 5 and 8 birds, three pullets of each family were selected by means of a table of random numbers, or 12 pullets for the set as a whole. Since the population was not culled at any time prior to the beginning of laying, the pullets may be considered to represent a random sample of the matings analyzed. Of the 31 sets, 13 had 3 pullets per family, 7 had 4, 10 had 5, and 1 had 6, involving a total of 548 pullets. Thus while the number of birds varied between sets, within each set the number per family was constant.

This characteristic of the data permitted the carrying out of analyses of variance within each set so that the total variance could be partitioned into four parts: (1) due to differences between sires, (2) due to differences between dams, (3) interaction, and (4) error. The first of these unfortunately represents not only the genetic contribution of the sire to the character of the daughters, but also the inter-year variability, since the two are perforce confounded in this design. The second provides a measure of the dam's contribution, although should persistent maternal effects exist they would be included in this portion of the variance. Outside of the unlikelihood of such a situation such an eventuality would only lead to an overestimate of the dam's genetic contribution to the daughters, but not affect the primary problem, that of nicking. The existence of the latter can be detected by the magnitude and statistical significance of the third of the listed sources of variance.

An additional point needs to be mentioned, and that is the fact that some of the sets include matings between individuals from populations not interbred for several generations. These matings were made in various inheritance studies in which the F_1 birds were eventually discarded without having reproduced themselves. Such sets would obviously have a greater likelihood of exhibiting nicking than sets derived exclusively from freely interbreeding populations.

RESULTS

As already stated the magnitude of the interaction in excess of the errors in the above described analyses of variance may be taken as a measure of the import of nonadditive action or more specifically of nicking. The relatively small number of birds in each set of data may be a limiting factor in the verification of the statistical validity of any interactions present. It is possible to circumvent this difficulty by obtaining a pooled estimate for all the sets of matings studied, provided their variances are homogeneous. A somewhat similar block design has been used by Lush and Molln (1942) in analyzing litter size in swine. A simple test for homogeneity has been proposed by Bartlett (1937) and applied to data at hand in accordance with the procedure outlined by Roessler (1943). The χ^2 for the 31 sets with 30 degrees of freedom was found to be of a magnitude (67.49) indicating some amount of heterogeneity between the sets. Examination of the values contributed by each of the sets to this χ^2 revealed three sets to be responsible for a large share of it. When these three sets were eliminated the recomputed χ^2, for 27 degrees of freedom was found to be 31.83, corresponding to a P point in the neighborhood of 0.25. Thus the 28 remaining sets may be considered to have homogeneous variances permitting valid pooling, while the data for the other three sets may be considered separately.

TABLE 1

Pooled Variance of Homogeneous Matings

	Total	Between sires	Between dams	Interaction	Error
Degrees of freedom	464	28	35	35	366
Mean square	598	1974	921	498	471
Percentage of variance ..	100.0	24.2	7.6	0.9	67.3
F		3.96	1.85	1.06	

Table 1 presents the pooled variance due to each of the indicated sources and clearly shows that no significant interaction is present in the material at hand; or in other words that nicking does not play an important part in the determination of sexual maturity. This is further confirmed by the fact that none of the contributing sets, whatever their origin, had interactions that were of significance. The variances of the three sets that were not used for the pooled estimate are presented in Table 2. It is clear from the readily computable variance ratios that nicking is of no greater significance in these matings than in the other 28 sets.

To the knowledge of the writer only one previous analysis of nicking in animals with results capable of statistical verification has been presented. Seath and Lush (1940) by a different method reached the conclusion that nicking is of little importance in the inheritance of both milk yield and butterfat percentage in dairy cattle. Attention must also be drawn to their *a priori* arguments against any widespread effect of epistatic deviations. The data presented in this paper bear out this point of view (though it should be noted that contrary argumentation has also been reviewed by Seath and Lush). It is of course, impossible to generalize from these results to other characters, but with respect to this population, it may be asserted with some confidence that epistatic deviations play no important rôle in the determination of sexual maturity. Consequently, once a breeding bird has been proven with one mate or a series of mates, it may be considered to possess genotypic qualities desirable in whatever mating it is used.

TABLE 2

VARIANCE OF MATINGS CONTRIBUTING TO THE HETEROGENEITY OF POOLED DATA

Set number		12	15	18
Total	degrees of freedom	11	19	23
	Variance	1,512	3,483	1,653
Between sire means	degrees of freedom	1	1	1
	Variance	2,324	22,178	1,219
Between dam means	degrees of freedom	1	1	1
	Variance	19	12,694	950
Interaction	degrees of freedom	1	1	1
	Variance	126	6,193	425
Error	degrees of freedom	8	16	20
	Variance	1,771	1,570	1,771

Reference should be made here to an investigation of a similar problem in corn by Sprague and Tatum (1942), who found that in unselected material differences between lines were largely due to additive effects of genes ("general combining ability"), while nicking ("specific combining ability") was of no relative importance. However, in crosses between lines already selected for high general combining ability, non-additive or nicking effects assumed a much greater rôle. The process of selection resulted here in the elimination of the differences in additive effects between lines, so that specific combining ability became responsible for the major proportion of the genetic differences between the selected lines. Presumably the same situation may arise in the case of sexual maturity in poultry, should internally homogeneous lines similar to each other in phenotype but differing in genotypes be established.

ESTIMATES OF INDIVIDUAL HERITABILITY

The partitioning of the variances within sets of matings presented in pooled form in Table 1 permits the estimation of the degree of individual heritability of sexual maturity. Previously (Lerner and Taylor, *loc. cit.*) the degree of family heritability (the portion of variance between families of full sisters which is attributable to genetic effects) has been estimated at about 20 per cent. Certain features of the present design referred to in its description place a definite limit on the precision of the estimate possible here. However, a rough appraisal of genetic variability can be made from the intraclass correlations appearing in the form of percentage of variance in Table 1. Assuming the absence of sex-linkage and of non-hereditary maternal effects, twice the variance attributable to the dam plus that attributable to interaction yields a total heritability of about 16 per cent. On the other hand, if the variances attributable to all sources except the error are added up an estimate of the genetic variance between individuals approaching 33 per cent. is obtained. While the former figure is a definite underestimate in view of the demonstrated sex-linked differentials (Lerner and Taylor, *loc. cit.*), the later is undoubtedly an overestimate, since it includes the variation between years confounded with that between sires. If the purely hypothetical maternal effects are considered to be at the least offset by sex-linked effects the probable limits of individual heritability of sexual maturity are between 16 and 33 per cent., a range covering the previous estimate of family heritability.

SUMMARY

An analysis of diallel matings in a White Leghorn flock indicates that "nicking" or non-additive deviations in general do not play an important part in the determina-

tion of sexual maturity. The individual heritability of this trait is found to be within the broadly established limits of 16 to 33 per cent.

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HEAT-INDUCED STERILITY AND ITS POSSIBLE BEARING ON EVOLUTION

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WHEN viewing the events that transpired during the course of evolution as portrayed by the paleontological record, we are confronted by the notable frequency of extinction of families, genera and species, and to a more dramatic extent by the phenomenon of the entire disappearance of many apparently virile and even once dominant faunas. The nearly complete obliteration of the reptiles as represented by the archosaurians, and the failure of many families and genera among the mammals, birds and other animal organisms furnish typical illustrations of these events.

Extinction has been explained as a possible result of orthogenesis culminating in the over-development of harmful traits; more recently it has been explained as a simple accessioning, or accumulation of deleterious mutations, or as being due to racial senescence, or as a result of unaccustomed stresses brought about by any one of several new biotic complications. Changes in climate have been proposed as possible explanations of extinction in certain groups, the most recent one being that of Cowles (1939 and 1940) advocating high temperatures as a possible factor in the case of the late-Cretaceous archosaurians.[1] Wieland (1942) has objected to this view and has proposed that changes in atmospheric carbon dioxide content brought about their demise due to strangulation, resulting from loss of the necessary respiratory stimulant!

Although there is evidence to support some of these hypotheses, and although several of them, either by themselves or in combination, may have been involved in some instances of extinction, none is completely satisfactory and it seems desirable to call attention to a commonly known characteristic of terrestrial animal organisms that may ultimately lead to the inclusion of another important factor involved in the extinction of faunas and the general process of evolution. At present the evidence is fragmentary, and promises to remain so until attention is directed toward the solution of the many problems that seem to contain the necessary information. These problems are too numerous to be solved in a short time unless many individuals of diversified training can be induced to contribute their knowledge and the results of their studies.

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[1] I am greatly indebted to Dr. Edwin H. Colbert, of the American Museum of Natural History, for the only tenable objections to the original heat theory. His objections and my persistent conviction that high temperatures were somehow involved in the phenomenon of extinction led to the present modification of the original theory.

The animal trait that may have played so significant a role in evolution is the susceptibility of the male germ cells, at least during active spermatogenesis, to temperatures above normal for the testis. This temperature response is modified by both intensity and duration of the exposure and produces varying effects on fertility by causing sterility or by diminishing the numbers or viability of sperms. As will be seen, there is also the possibility that high temperatures may be connected with the proliferation of variations.

If the prehistoric terrestrial animals responded to overheating in the same manner as those of today, there can be little objection to the suggestion that testicular susceptibility to supra-normal temperatures might have been a significant factor in the process of evolution. Obviously, there is no direct method by which this can be positively ascertained—therefore only a tentative conclusion can be achieved by the deductive method, and this requires the gathering of pertinent evidence from as many and diversified sources as possible. Nonetheless enough information seems available to provide the general framework necessary for a preliminary exposition of a tentative hypothesis.

A review of the literature provides ample evidence for the statement that heat-induced sterility at the present stage of evolution, is a phenomenon of wide occurrence and that it characterizes every known species of terrestrial animal that has been studied from this standpoint. Although lowering temperatures to below normal may result in a temporary suppression of spermatogenesis, this appears to be a result of a general reduction of the metabolic rate, and the effects are evanescent. Normal low temperatures are not believed to play a major part in the reproductive processes because of the fact that most, if not all, cold-blooded animal organisms operate at maximum efficiency at thermal levels that are only slightly below the threshold where heat induced damage is inevitable. On the contrary, with the possible exception of the stenothermal warm-blooded animals, the danger point from cold lies well below optimum thermal level (Cowles, *supra cit.*).

The universality of the phenomenon of testicular sensitivity to heat is readily apparent from even a casual survey of the pertinent literature.

Young and Plough (1926) have noted the phenomenon of heat induced sterility in *Drosophila,* while Norris (1932) reports a similar condition in the moth *Ephestia kühniella.*

The effects of cryptorchidism and heat on the mammalian testis were partly surmised at the time that Felizet and Branca (1898 and 1902) called attention to the effects of cryptorchidism in man. Sand (1922) experimented with surgical replacement of the testes and found that degenerative effects resulted, but it was not until the studies of Moore (1924) and Moore and Chase (1923) and the classical work of Moore and Oslund (1924) that heat-induced sterility came to be a widely recognized fact.[2]

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[2] Since the writing of this paper, my attention has been called to the report of J. K. Lamar and R. Rogers, 1943, "Season and Human Fertility in Galveston, Texas." (Abstract, *Anat. Rec.*, v. 87, 4) in which it is shown that there is a correlation between low temperatures and the rate of conception. More notable because of the absence of the complicating factor of the psychological

These and other experiments on mammals, such as those of Harrenstein (1928), Young (1929), Phillips and McKenzie (1934), provide indubitable proof that heating the mammalian testis to above its normal temperature will cause sterility, and that in the scrotal mammals, even normal body temperatures applied to the testis cause its degeneration. These studies, with those of Moore and Quick (1942) have provided unquestionable evidence that the chief physiological function of the scrotum, and testicular migration, is thermoregulatory. The extraordinary significance of these findings in terms of survival and evolution have not been commented upon, possibly because at the time they seemed to represent only an isolated phenomenon.

Riley (1937) has presented strong evidence for the existence of what actually amounts to periodic heat sterility in the English sparrow. In this bird, spermatogenesis is an interrupted process which takes place only when the body temperature falls several degrees below normal in the early morning hours. Under conditions of normal daytime activity, the high body temperature appears to inhibit spermatogenesis. It is probable that this interrupted type of spermatogenesis may have been anticipated by the reptiles.

Witschi (1942) has called attention to the remarkable affects of temperature on the ovo-testis of larval amphibia and while these findings do not constitute direct proof, they do suggest the possible presence of special germinal sensitivity and the need for experimental studies to determine whether or not thermally induced sterility may characterize the adult Salientia. Grobstein (in lit.) states that the testes of *Triturus rivularis* collapse prematurely if the animals are maintained in warm water in the laboratory, whereas under cold conditions they re-

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element, is the correlation of high temperatures with the increase in stillbirths resulting from conceptions occurring in July, August and September, which may increase as much as 134 per cent. above the minimum from conception occurring in June. The studies are being continued.

main normal for a longer time. This suggests the possibility that careful investigation will disclose the existence of gonadal heat collapse in these animals.

Experiments have been completed on one species of reptile, the only remaining group of terrestrial vertebrates for which there are apparently no reports on the sterilizing effects of high temperature. These recently concluded experiments (Cowles and Burleson, in ms.) have produced indubitable proof that non-lethal high temperatures (36.5° C.) will cause sterility in the nocturnal lizard *Xantusia vigilis*.

Pertinent observations on the reproductive habits of the garter snake *Thamnophis sirtalis sirtalis* have been recorded (Blanchard and Blanchard, 1942) and the report indicates that these reptiles also may be susceptible to heat effects since fertile matings do not usually occur after the onset of hot weather during late spring; that is, ordinarily not after mid-May. It is therefore highly significant that a period of cool autumn weather followed by a libido-stimulating rise in temperature not only results in renewed matings, but that these are fertile. Apparently even the viable sperms of late spring fail to survive the summer months, whereas those produced in the autumn will remain unharmed by the low winter temperatures to which they are subjected.

Although I have been unable to find conclusive reports on the fishes, there seems to be a strong possibility that even these non-terrestrial organisms may suffer germinal impairment by exposure to heat.

From the foregoing review of the effects of high temperature on the male reproductive mechanism of a wide variety of terrestrial animals, it appears possible that most representatives of all the Classes of land-dwelling organisms also may be characterized by the existence of this curious thermal hiatus. It is particularly notable that in at least one group, normal temperature requirements are greater than can be endured by the spermatogenic process.

While it may be protested that chance has led to the selection of special cases, actually there is little possibility that the wholly random selection employed in these studies favors an accumulation of artificial evidence. It would seem, on the contrary, that these examples could not have been selected in a less prejudiced manner, and that therefore they should be thoroughly representative of the conditions that we may expect to find in most of the species of classes and orders of land animals.

Although there is no way of knowing whether the ancestral representatives of these orders were characterized by this thermal hiatus (which might be known as thermal divergence or thermal incompatibility) it is highly improbable that so great a diversity of types as those represented in these studies could have developed the trait independently. Certainly it is possible that the existence of the character in living representatives of animals of very diverse nature but having common ancestral origins suggests the possibility of its great antiquity and thus provides us with the tentative basis necessary for the assumption of its phylogenetic spread and continuity over a long period of time.

The circumstances that might result in overheating the body, and thus the testes, most obviously would be a change in climate that would produce either a higher mean temperature throughout the year, or higher mean or maximum temperature during the period of active spermatogenesis. Changes in the relative humidity might possibly lower the efficiency of the cooling mechanism to such a degree as to allow an injurious rise in body temperature, but this appears to be rather less probable than a rise in environmental temperature. Any of these environmental changes presumably would permit the survival of only those forms that became accommodated to the new conditions through successful modification of habits, as by the adoption of nocturnality or migration, or by a change in habitat as, for instance, by retreat underground or to an aquatic environment. It is difficult to conceive of any of these changes resulting by any means other than the process of mutation channeled by selection through survival.

The problem of higher environmental temperatures might also be met by the development of body cooling devices; sweating, panting, etc.—such as those found in the mammals, or by some analogous device such as the air sacs that almost invest the avian testis. In the absence of effectual body-cooling strategems, the testes may be separately cooled during spermatogenesis by placement in cooler areas in the abdominal region, or by means of this periodic migration to just beneath the skin or by permanent suspension outside of the body, in a thermoregulatory structure, the scrotum. All these devices are found in the modern mammals.

In the absence of effective temperature protection for the testes, some other expedient may be necessary and it is entirely possible that the almost universal habit of spring mating by temperate zone cold-blooded land animals, and the warm-blooded birds and many of the mammals, may be a contrivance for avoiding the fecundity limiting effects of over-heated germ plasm. Although other ecological factors are undoubtedly involved in the matter of spring breeding they have apparently obscured the possibility that high temperatures may provide at least a partial alternative, or often an entire explanation of the phenomenon. Support for the thesis that spring breeding may often be due to thermal compulsion can be drawn from the studies conducted on the breeding habits of the thirteen lined ground squirrel, *Citellus tridecemliniatus* (Wells, 1935, a and b) and the reproductive habits of the garter snake (*supra cit.*) and other instances of otherwise puzzling seasonal mating behavior. Undoubtedly many other similar examples will be found when investigation is directed toward their discovery.

Although it is entirely possible that in the past there may have been periods of high temperature comparable to the intervals of subnormal thermal conditions that resulted in glaciation, these interludes would not leave such gross and relatively easily interpreted evidence of their occurrence as those which characterize areas subjected to the action of massive ice movements. Because of the difficulty of the problem of finding geological or other direct evidence of high temperature it is fortunate that no matter how plausible the future evidence may be, it is not necessary to require the establishment of high paleoclimatic temperatures in order to explain how heat damage could have been incurred by male reproductive organs.

The alternative non-environmental changes that might result in heat-induced lowering or fertility are many. In the habitats of almost all, possibly all, of the cold-blooded organisms, the heat of the sun's unmodified spring and summer radiation is so great that it will kill some of the cold-blooded animals including reptiles in a matter of hours at most, quite commonly in minutes. This is highly significant since we are now living in an admittedly semi-glacial period rather than an even equable one. Under the either equable, warm or possibly hot epochs when corals, palms and other supposedly tropical organisms flourished as far north as Alaska and as far south as Tierra del Fuego, our temperate zone might have been considerably warmer than now. Therefore, judging by the rapidity with which reptiles may be heat-killed now it seems possible to draw the conclusion that although many of the cold-blooded animals have access to temperatures considerably higher than those they are accustomed to utilize, and might be capable of adopting them so far as the body cells and environmental requirements are concerned, they have not been able to do so in part at least because of the limitations imposed upon them primarily by their heat-sensitive germ cells and other associated requirements.

Birds and mammals, receiving most of their heat from metabolic activity, and having fairly effective heat control mechanisms, are not so apt to be subjected to environmental changes as are the so-called cold-bloods, the ectothermic types; nevertheless, the warm-blooded or endothermic animals possess within themselves the capacity for generating higher than normal temperatures. Proof that they frequently do so for short periods is evidenced by fevers; furthermore, the loss of effective cooling devices, as for instance sweating, or changes in the environment which would lessen the effectiveness of these mechanisms might also result in the development of high temperatures. That the body cells can withstand these upward changes within certain limits is well known, as is the fact that prolonged or very high fevers can produce at least temporary sterility in man. In so far as the mammals are concerned, it is clear that probably most of them could not adopt even the avian thermal level without danger of serious disturbances or impairment of the male reproductive processes, that is, unless the change was accompanied by the necessary succession of mutations that would apparently be needed to produce a compensating increase in the effectiveness of the testicular cooling mechanism, or which might create some other coordinating changes. The effects of over-heating in birds does not seem to have been studied from this point of view although there is some encouragement from indirect evidence bearing on this problem (Kendeigh and Baldwin, 1937).

If it can be discovered that there are valid objections to the preceding suggestions as to how overheating may have taken place, it still will be necessary to consider the possible temperature adjustments that must have been made as the animals passed through the various transitional stages leading from their original aquatic habitats to their final conquest of the land and their ultimate triumph over a thermally changing environment. Certainly it seems logical to suppose that the emergence of the vertebrates from water to land must have exposed them to rather violent changes in insolation and thus have confronted them with the necessity of solving new thermal problems.

The change from the water-evaporating skin necessary for amphibian respiration, to the dry skin of the more and more water-independent reptiles has certainly resulted in rather drastic upward revisions both of their temperature tolerance and requirements. In a similar manner, the development of fur, feathers and, in some cases, the additional insulation provided by the acquisition of massive layers of hypodermal adipose tissue, together with the appearance of internally produced and conserved heat would have created new thermal hazards. It is curious, in view of its importance, that there are apparently no special sensory nerve endings having the express function of protecting the testes against heat damage.

Each of these radical evolutionary steps must have been accomplished by the development of compensating advances in the toleration of heat by all of the body cells, both somatic and germinal, otherwise overwhelming heat stresses would have been imposed upon both, but especially on the germ cells because of their probably greater susceptibility to heat. It would seem reasonable to suppose that many of the now non-existent forms simply may have failed to make the necessary adjustments, which therefore would mean that the survivors are a product of successful innovations, beneficiaries of fortuitous mutations which happened to take one or more of the only courses of thermal adjustment that would make survival possible.

If the mutational process is accepted as the necessary mechanism for providing the variations requisite for the operations of selection, then these mutations and the accompanying changes would necessarily have had to bring about a simultaneous and coordinated alteration in the temperature tolerance of both the body and the germ cell mechanism, and whatever the reason, this apparently has not been perfected in any of the forms that have been studied. Because of this situation the question naturally arises as to the nature of the difference in heat tolerance of these two groups of cells; that is, are they mutationally independent of each other?

It would seem from available evidence that the answer to this question is not at hand, although additional studies on thermal divergence may provide the answer. Even if the cells are not independent, the male germ cells nevertheless are more vulnerable than body cells to the effects of excessive heating. This may be because of the nature of the processes involved in maturation of the germ cells; on the other hand, evidence may arise to show that the body and germ cells are in a sense mutationally independent and separate. Whatever the answer may be, it is not necessary to dispose of this problem before it is permissible to consider other aspects of the well established fact of the thermal vulnerability of the male germinal tissue.

One of the most interesting possibilities inherent in thermal divergence of male germ cells results from the advantages accruing to an animal organism whose body cells are capable of operating at the accelerated metabolic rate resulting from higher temperatures. Van't Hoff's law of a doubling of metabolic activity with each 10° C. rise in temperature is pertinent here. Survival of an organism is often dependent on its alertness to danger, and its speed in avoiding predators—similarly the predators themselves profit by reason of slight advantages in speed and alertness. Since higher temperatures accelerate all physiological activity, survival can conceivably depend on this factor as well as on purely morphological adaptations leading to greater speed.

There would seem to be a strong possibility that survival of the body might frequently depend on the small additional margin of speed and alertness that should result from a utilization of a slightly higher body temperature, but that progress in this advance by a species would be penalized or prevented by the thermal limitations imposed by the need for viable male germ cells. If this can be established, it would seem to be a remarkably significant example of such conflicts of interest.

The compromises resulting from the conflict between the advantages accruing to an animal capable of adopting a higher temperature and the attendant diminution in fecundity resulting from the instability of the germ plasm might be an important factor in accelerating the process of evolution. This is especially significant because Plough (1942) has shown that high temperatures may increase the mutation rate in *Drosophila* by five times the normal, and has pointed out the significant increase in the number of lethals which usually accompany mutational changes. In his concluding sentence he says, "Where mutation is the limiting factor, it may well be that the increased mutation rate at a higher temperature would produce marked increases in evolutionary diversification. It is perhaps this relation of temperature to mutation which determines the greater number of species in tropical areas."

As has been shown in the papers cited earlier in this discussion, it is clear that there is another, possibly more exaggerated, impact of temperature on an organism; namely through heat produced sterility or at least a reduction in fertility where the exposure is not so great. With these two effects of temperature in mind, there can be little doubt that the necessary number of replacements left by an animal can be drastically diminished by one or the other or both of these heat effects. Whatever the cause of a reduction in fertility, there can be little doubt that the delicate adjustment between degree of fecundity and the required number of offspring necessary for survival of a species at even normal mortality rates, will be seriously disorganized.

It would appear to be an inescapable conclusion that a disruption of normal population balance would reduce intra-specific competition by reducing population pressure. This initial effect might be accentuated still more through the loss of, or damage to, the essential reproductive stimulus inherent in a normal population density. Reduction in population pressure would seem to provide for an increased play of mutations; i.e., there would be an enlarged scope for individual variation because of an unwonted increase in the amount of available territory and consequent changes in the nature of competitive activity.

Another product of a reduction in population might result in a radical change in the effects of predation, parasitism, and inter-specific competition. In either case it would appear probable that a higher premium would be placed on mutations leading to the production of adaptive changes that would be required as a result of alterations in the limiting and molding effects of population pressure.

Changes such as those mentioned and other concomitant phenomena might result in greater variety of opportunities for a greater variety of appropriate mutations, a highly important condition if *Drosophila* can be taken as exemplifying the effects of heat, either past or present, on rates of mutation.

The sum of these possibilities seems to indicate a theoretical trend toward rapid speciation, possibly accompanied by gradual decline of the original stock, and ultimate extinction or bare survival as a relic fauna. Very high or very suddenly applied temperature stresses would seem to favor outright extinction of one fauna, followed in especially fortunate successful forms by replacement with another group originating from some less specialized ancestral stock.

From the conclusions of Plough (*supra cit.*) regarding the effect of heat as an accelerator of mutations, it would seem reasonable to expect greater plasticity in a stock in which the compromise between body cell versus germ cell temperature requirements was toward the adoption of higher temperatures, while conversely, it would be expected that heat-conservative types, climate permitting, might incline toward stability. While this may be an oversimplified statement of the problem, it may contribute toward an explanation of the multiplicity of species in the tropics and scarcity of species in cold areas, but it should be noted that this explanation is diametrically opposed to the conventional explanation as presented by Hesse (Hesse, Allee and Schmidt, 1937.)

Many otherwise puzzling biological phenomena can be explained readily on the basis of this hypothesis, or at least it may be said that the phenomena fit into the hypothesis. Some of these problems have been discussed previously in terms of the effects of heat on the somatic cells; i.e., the bodies of the organisms (Cowles, 1940), but in most instances the present modification and elaboration of the original idea is even better suited to the demands of the evidence.

In the original account, such phenomena as extinction of the dinosaurs and possibly other cases of extinction as well, the migration of birds, development of nocturnalism, etc., were tentatively explained as a possible result of the effects of heat on the somatic cells. While these seem better suited to an explanation based on thermal divergence, some of the other phenomena, such as the nature of the circumstances leading to adoption of terrestrial habits by the original land-inhabiting vertebrates, the transformations from amphibian to reptilian types and so forth, might be modified to include the stimulating and accelerating effects of heat on the somatic cells as well as the limiting effects of heat on the germ cells.

It is reasonable to expect the discovery of many instances in which the germ cells will equal the temperature tolerance of their somatic vehicle. The present absence of known instances of thermal harmony is most surprising and is not the least suggestive part of the picture. While discovery of harmonized cellular tolerance will not be "the exception that proves the rule," the occurrence will not invalidate the argument that thermal divergence constitutes an exceedingly important factor in evolution.

SUMMARY

The advantages accruing to an animal having a higher

than normal temperature, in combination with the existence of a limiting heat sensitivity characterizing the male spermatogenic mechanism, has inevitably led to a conflict between interests which may have been resolved by changes in habits, habitat selections, or the development of general body cooling, or specific testicular cooling devices. Failure to solve the conflicting requirements may have led to either actual or virtual extinction. It appears probable that in many instances, there has been some compromise between opposing interests and that only minor heat strains have been incurred. If the effects of heat on *Drosophila* may be taken as an indication of historic thermal response of spermatogenesis characterizing all organisms, at some or all stages of development, we may logically expect that this thermal civil conflict expedited the process of evolution by accelerating the mutational rate.

The extinction of the archosaurians, also extinction of the primitive mammals and many other terrestrial animals, as well as many other phenomena apparently fit this concept of heat as a factor in the process of evolution.

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REVIEWS AND COMMENTS

EDITED BY PROFESSOR CARL L. HUBBS

IN these reviews and notices of current biological publications emphasis is given to books and major articles which fall within the special scope of THE AMERICAN NATURALIST, in that they deal with the factors of organic evolution. REVIEWS AND COMMENTS are meant to include also such general discussions, reports, news items and announcements as may be of wide interest to students of evolution. Except as indicated, all items are prepared by Dr. Carl L. Hubbs, Scripps Institution of Oceanography, University of California, La Jolla, California. All opinions are those of the reviewer.

Foundations of Plant Geography. By STANLEY A. CAIN. New York and London: Harper and Brothers, 1944: i–xiv, 1–556, figs. 1–63. $5.00.

As EDGAR ANDERSON has wisely said, Cain has brought together the stones but has not built them into a foundation on which a superstructure can be laid. Biogeography remains a mass of largely unorganized material. Perhaps some master mind can synthesize the fundamentals of a science of the geography of organisms. More likely, however, this accomplishment will evolve from the labors of many workers, a few of whom will contribute important constructive suggestions. Cain's compendium of facts and interpretations will render these labors easier and more likely to lead to synthesis. Its breath will go far toward making biologists in general biogeographically conscious. As a result more workers in more fields will consider the geographical implications of their findings, and will be more apt to publish such considerations either in a general section of their papers or in separate articles.

The science that should develop out of these distributional considerations will, it is hoped, be that of biogeography. Cain has obviously appreciated this need, for many of the examples that he gives are drawn from animal geography. Like genetics and evolution, biogeography should be a science bracketing the entire field of biology.

The variety of poorly synthesized fields that are basic to biogeography renders this a difficult book to review.

This difficulty is enhanced by a text that at times grows heavy, by some inconsistences in organization, and particularly by the ecologists' bad habit of using an unfamiliar jargon that is both literally and figuratively "Greek" to most biologists. One may wonder whether ecologists may not be glossing over a deficiency of fundamental contributions by a veneer of highly technical terms. The new term "areography" may be objected to not only for its novelty and its similarity to aerography, but also because it is of bastard origin and denotes a concept that is sufficiently well designated by "biogeography" and by "chorology."

Paleoecology appropriately is the first of four headings under which the "foundations of plant geography" are treated. Why the chorological aspects of paleontology are not included as such is not evident. Certainly the past distribution of organisms in space even when empirically determined, has a bearing on present distributions. Such data are of course considered by Cain. He probably adopted the section heading of paleoecology because he himself is primarily an ecologist and because he wanted to emphasize the important biogeographical bearings of this new phase of paleontology. In this aim he was successful. The presentation of paleoecological data and interpretations constitutes perhaps the most useful feature of the book for students of biogeography and speciation. For instance, we have here what I understand to be the best summary of Mason's important researches on the Cenozoic history of the Californian closed-cone pines. "Pollen Analysis as a Paleoecological Research Method" is another very informative chapter.

The main body of phytogeographic data are marshalled under "Areography." Most of the interpretations in this section are characterized by a judicious objectivity rather than by a taking of sides on debated questions. For instance, in dealing with the controversy of whether primitive forms occur at the center or at the periphery of the range, his attitude is conservative and I think wise, for logic as well as facts confirm his view that such forms may now occupy either position, or neither. Abundance of forms is not taken as an invariable or reliable index of center of origin, for "a phyletic stock that has had its center of origin elsewhere may, through migration, encounter a region in which there are numerous available ecological niches that are unsaturated, that is, in which competition pressure is low. Such a region may provide a variety of habitats with at least partial isolation. Under such conditions a phyletic stock may show a 'burst' of evolutionary adaptation." That has the ring of sound speciational philosophy.

As exemplified by this quotation there is much discussion of speciation in the section on "areography" but the main treatment of the species problem is given under the third heading, "Evolution and Plant Geography." Here also is the discussion of such topics as isolation, species stability and rate of evolution. It is concluded that speciation may be very rapid or very slow.

A single genetic aspect of the species problem, namely polyploidy, is selected as the fourth and final section. Unhappily, there is no concluding or summarizing section.

One of the main reasons why this treatise will stand in many working libraries on the shelves allotted to often-used books is the bibliography of 720 cited references: an ample evidence of the long and devoted scientific labor that went into the production of this outstandingly useful volume.

NOTICES OF NEW BOOKS

Fact and Fiction in Modern Science. By HENRY V. GILL. First American Edition. New York: Fordham University Press, 1944: 1–136. $2.50.—Originally published in 1939 in Eire, where it has gone through three printings, this series of essays by a scientifically trained Jesuit priest is now offered to the American public. Repeatedly throughout the book there is inserted a defense of the Catholic viewpoint. I hope that intelligent Catholic opinion is as broad as it is here said to be. In regard to evolution the opposition of the Church is indicated as being limited to its denial of the view that life could have originated through the action of natural physical laws. The increased organization that is inherent in evolution is held to violate the basic physical law of entropy. A vital principle is therefore indicated, the author argues, and the existence of God is proved. The question of what part of human evolution is attributable to natural laws is avoided. As would be expected, birth control and eugenics through sterilization are opposed. In examining such treatises many scientists not steeped in Catholic doctrine will be struck by the inconsistency between a real scientific approach toward most problems and the inflexible conviction that certain documents and agencies are infallible, even when the claim for infallibility is restricted to a minimum of philosophical fundamentals and to certain applications of science to human affairs.

A Shorter History of Science. By WILLIAM CECIL DAMPIER. Cambridge: At the University Press; New York: The Macmillan Co., 1944: i–x, 1–189, pls. 1–9, figs. 1–14. $2.00.—The author of the great "A History of Science" has done science a new service in rewriting his major treatise in abbreviated and somewhat popularized form to make more widely available the fundamentals of this important subject. Modern science is clearly portrayed as an immense field, potent in its bearing on all phases of human culture. The accompanying plates are unfortunately drab, for they are printed on soft text paper. It is to be hoped that a more attractive edition will be produced when wartime restrictions permit.

The Book of Naturalists. An Anthology of the Best Natural History. By WILLIAM BEEBE. New York: Alfred A. Knopf, 1944: i–xiv, 1–500. $3.50.—An accomplished naturalist as well as an able writer, William Beebe was particularly well qualified to select and to annotate the material for this anthology. The selections are good and the annotations are illuminating, though, of course, others would have made different selections and might have prepared more detailed annotations. It is very doubtful, however, whether any one else would have compiled and condensed the gems of natural history writing of all time into a volume more likely to attract and hold the interest of general readers. Classical and foreign-language essays are presented in good English translations. Part I presents the production of early naturalists, from an "unknown Magdalenian" cave painter, Aristotle and Pliny down to Thoreau. The second part samples modern natural history writings, from Darwin, Wallace, Agassiz and Huxley to Rachel Corson.

That Vanishing Eden: A Naturalist's Florida. By THOMAS BARBOUR. Boston: Little, Brown & Co., An Atlantic Monthly Press Book, 1944: i–x, 1–250, 15 pls. $3.00.—As a source of pure intellectual joy there is almost no match for the experiences of a master field naturalist in an unspoiled land, rich in wildlife; for depths of regret there is little to equal the feelings of such a naturalist on the rapid despoliation of a land and biota that he has studied and loved. These joys and these regrets, vividly portrayed, are the theme of this altogether fascinating sequel to "A Naturalist at Large." The book appropriately starts with "The First to Arrive": the Miocene, Pliocene and Pleistocene Faunas, in the reconstruction of which the author has played a leading hand. Primordial Florida is then pictured through the highly regarded observations of William Bartram. Earlier human occupation is hinted at in the chapter on mounds. There is then given a sympathetic account of the Seminoles, which has been criticized as indicating a too complete loss of the aboriginal culture of these Indians. A very few other errors, excusable in the light of the tremendously broad and generally accurate nature wisdom of the author, were noted—for example, the statement that *Leptolucania ommata,* one of the oviparous cyprinodonts, is viviparous. Successive chapters deal with the Florida forests and the dire part that fires have played in their destruction; with delightful camping experiences; with beaches and sponges, springs and lakes, and mammals; with ditches, nature-destroying though often filled with a concentration of aquatic life; with "The Garden of Eden," alligators and crocodiles, the islands and the keys. The reader is then escorted to the "kampong" of David Fairchild, another grand naturalist whose world-wide wanderings have become increasingly confined to Florida, and to the Fairchild Tropical Garden. Final chapters deal with the Everglades National Park and with the future of Florida wildlife. The latter part of the book is written in a note of subdued optimism, as the author hopefully points out means by which the vast remaining natural history resources of our most nearly tropical state may be salvaged and utilized.

The Conservation of Natural Resources. A textbook for Junior and Senior High Schools. By H. BASIL WALES AND H. O. LATHROP. Chicago: Laurel Book Co., 1944: i–viii, 1–554, 191 figs., 5 maps, 7 charts. $2.00.—There are few needs so fundamental to our national future as is early and proper instruction in conservation. The lack of suitable texts has long deterred the filling of this need. For this reason the publication of this excellent book is an event of great potential importance.

In general the text is appropriately written down for senior high schools, though perhaps hardly for junior high schools. It should be suitable also for a non-professional college course in conservation. The authors have judiciously selected and balanced the material, though what P. S. Lovejoy termed the saw-log point of view is perhaps overstressed. Many preservationists and lovers of nature will flush over the suggestion "that the waste of timber in natural parks cannot be tolerated."

Such fundamentals as forestry, soil conservation and wise water utilization are particularly well treated, and it is a happy thought that this book may help to bring about a wiser public opinion and a stronger pressure for sane land management. Other phases of conservation are well covered; none of importance, except human conservation, is left out.

The factual basis is unusually sound, though statistical data are largely avoided. In parts perhaps too much detail is included for the purposes of a high school text. Inadequate and erroneous statements seem few, though not wholly lacking. Thus the suggestion that the depletion of fish in the Great Lakes has not been as serious as in smaller waters gives a very poor idea of the seriousness of the depletion of commercial fishes in the inland seas. Fishery workers will also be surprised at the statement that the marine and salmon fisheries are regulated by the federal Fish and Wildlife Service. Such minor inadequacies and errors could readily be eliminated in future editions. They do not deter us from saying, Well done, Wales and Lathrop.

Aquatic Plants of the United States. By WALTER CONRAD MUENSCHER. Handbooks of American Natural History, 4: Ithaca, New York: Comstock Publishing Co., 1944: i–x, 1–374, figs. 1–154, maps 1–400. $5.00.—As is characteristic of the Comstock publications, this new handbook is authoritatively written, thorough in scope, an important contribution to science as well as to education. It is primarily a manual for the specific identification of the submersed and emersed aquatic plants of the fresh, brackish and marine waters of the entire United States. For this purpose the impressively clear-cut line drawings and the simple keys will serve well; often, however, only when flowers or fruits are at hand. The special emphasis on seeds and seedlings is an important feature. Two main criticisms occur to the reviewer. Firstly, the general introduction is tantalizingly curtailed. Secondly, the distributional maps, though a highly commendable feature, are not as instructive nor as neat as they might be. The major aquatic environments are poorly represented: less than a dozen each of lakes and streams are shown. With few exceptions there is given only a single record for any one state, and the placement of the dot is not consistently either the point of collection or the state center. The variable size and shape of the dots somewhat mars an otherwise very attractive presentation. These are, of course, minor criticisms. Muenscher has provided a very fine and useful handbook.

Birds of the Southwest Pacific. A Field Guide to the Birds of the Area between Samoa, New Caledonia and Micronesia. By ERNST MAYR. New York: The Macmillan Co., 1945: i–xix, 1–316, col. pls. 1–3, figs. 1–16. $3.75.—One of the minor tragedies of the Pacific operations has been the generally unanswerable call of zoology students in the war service for literature on the groups of animals in which they are interested. Their experiences would have been much richer, their life happier and their chance of making scientific observations or collections brighter, had books on the natural history of the region been available. For one group in one area, the birds of the Southwest Pacific, this need has now been filled, somewhat belatedly it is true. It is fortunate that the outstanding authority in the field has prepared this attractive book, which is well designed to meet the needs of the war-engrossed naturalist traveler.

Wildwood Wisdom. By ELLSWORTH JAEGER. New York: The Macmillan Co., 1945: i–xix, 1–491, pls. 1–193 and other figs. $2.95.—This "veritable encyclopedia of woods lore" is based largely on the expert woodcraft of American aborigines and early explorers but is made applicable to the needs of present-day wilderness travelers. As the product of a long historical study of Indian lore, supplemented by frequent periods of living among the natives and by close contact with leading naturalists and outdoorsmen, it portrays the means of comfortable existence while living off the country in north woods, arctic and desert, without recourse to "the myriad jingle-jangle gadgets of some of our modern outdoorsmen." Hundreds of items of woods lore and of woodcraft are ably illustrated by the author's own clear and clever sketches.

The Problem of the Derivation of the South American and African Fresh-water Fish Faunas. By WILLIAM A. GOSLINE. An. Acad. Bras. Ciencias, 16, 1944: 211–223.—Following a brief statement of diverse theories that would explain the similar biotas of South America and Africa and a critique of the ichthyological evidence, Gosline concludes that "the routes by which the Ostariophysi [the chief group of fresh-water fishes] of the southern continents became distributed cannot at present be profitably discussed. I hope the point is equally clear, however, that ostariophysine distribution does not fit the mammalian dispersal pattern." He then points out promising lines of further investigation. Such avoidance of convictions is more frequent in theory than in practice.

Boletim do Museu Nacional, Rio de Janeiro, Nova Serie, Zoologia.—The separate numbers of the new series of zoological papers issued by the Rio museum are appearing at gratifying frequent intervals. Numbers 13 through at least 29 were published during 1944. Among these papers particular mention may be made of two by BERTHA LUTZ on the biology and embryology of anurans. One (No. 15) deals with the direct development of an *Eleutherodactylus.* A larger publication (No. 17) treats the "Biologia e taxonomia de *Zachaenus parvulus.*" In Bulletin No. 24 AUGUSTO RUSCHI describes an intergeneric natural hybrid in the hummingbird family (Trochilidae).

SHORTER ARTICLES AND DISCUSSION

THE PRIMARY SEX RATIO IN DOMESTIC CHICKENS[1]

INTRODUCTION

So far as the writer is aware the primary sex ratio (percentage of males at fertilization) has not been reported for the domestic chicken. Mayr (1939) has reviewed the sex ratio in wild birds as far as known. It appears that there is a marked tendency for many species to produce an excess of one sex, usually the male. Landauer and Landauer (1931) summarized the data on chicks from a large number of workers on the secondary sex ratio (per cent. of males at hatching). These data include 67,993 chicks, of which 48.77 per cent. were males. Crew (1938) found no significant differences in the sexes with respect to embryonic mortality rate. Crew also reports the secondary sex ratio on 515,976 sex-linked chicks hatched as 50.34. On 2,216,051 pure-bred and crossbred chicks where sex was determined by the Japanese method the sex ratio was 51.38. The secondary sex ratio appeared to differ with the breeds studied. Landauer and Landauer (1931) are inclined to believe that the embryonic mortality rate is slightly greater in males than in females, although they recognize that data so far reported are conflicting.

THE PRIMARY SEX RATIO

In order to discover the primary sex ratio in the domestic chicken it is necessary to select females that show 100 per cent. fertility and 100 per cent. hatchabality and to accurately determine the sex of all chicks hatched. The period of observation may logically extend through March and April, which is the normal hatching period in this locality. The objective is to determine the sex of the chick from every egg laid.

In the ten-year period from 1935 to 1944 a total of 39 pure-bred Rhode Island Red females mated to pure-bred Rhode Island Red males qualified for study. Each female had a perfect record in fertility and hatchability and produced ten or more chicks during March and April. Since the sex of chicks was not determined until the age of eight weeks, there was a loss of 61 chicks upon which no sex record is available.

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[1] Contribution No. 546 from the Massachusetts Agricultural Experiment Station.

In Table 1 data are presented in summary form.

TABLE 1

SEX RATIOS OF HATCHED CHICKS

Year hatched	No. of dams	No. of chicks		Sex ratio	Sex not determined
		Male	Female		
1944	8	70	78	47.3	9
1943	4	43	45	48.8	9
1942	0	none		...	..
1941	4	51	47	52.0	3
1940	6	92	85	52.6	5
1939	5	50	48	51.0	10
1938	3	29	28	50.8	3
1937	2	22	22	50.0	4
1936	3	21	34	38.2	14
1935	4	54	51	51.4	4
Totals	39	432	438		61

In Table 1 the sex ratio of the 870 chicks whose sex was known was 49.7. In 1936 the sex ratio deviated widely from equality probably because of the large number whose sex was not recorded. By using the constant .527 recorded by Landauer and Landauer (1931) as the sex ratio of chicks dying between hatching day and eight weeks of age, it is possible to approximate the sex ratio of our total chick population. Simple calculation will add 32 males and 29 females to the totals in Table 1. The calculated grand totals became 464 males to 467 females which is as close to a 50 per cent. ratio as could be expected.

When only the 870 chicks whose sex is known are considered the sex ratio is 49.7. The standard error of mean difference between the yearly sex ratios and a 50–50 ratio was ± 1.42. These data again indicate that the primary sex ratio in Rhode Island Reds is 50–50.

In our data there were eleven hens with complete records for

TABLE 2

SEX RATIO OF CHICKS FROM INDIVIDUAL HENS

Hen No.	Chicks produced		Sex ratio
	Male	Female	
X296	4	14	22.2
X2670	4	7	36.4
T507	13	7	65.0
S2203	15	13	53.6
T2"52	18	12	60.0
S1042	9	9	50.0
R708	14	19	42.4
R1092	9	6	60.0
N2246	11	9	55.0
N2394	15	12	55.6
O2840	15	18	45.5
Totals	127	126	

sex of chicks produced. These are presented in Table 2 to show the extreme variability in individual families.

The data in Table 2 show that great variability occurs in the primary sex ratio between different families. This variability may be ultimately traced to gene differences. It is not at all unlikely that sex in domestic chickens may depend upon many genes and that many of these are located in the autosomes.

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INDUCED PARTHENOCARPY IN GINKGO[1]

THE occurrence of apomixis in its various forms is fairly common among the angiosperms, but so far as is known, is unreported among the gymnosperms. In some species of the angiosperms, apomixis is the usual method of embryo development and is genetically controlled, whereas in some other species, it appears to be facultative in the sense that it occasionally occurs normally in the failure of normal fertilization or it can be induced by various external stimuli such as x-rays, foreign pollen, chemicals, inadequate pollination, traumatic shock, etc. These facultative apomicts may be either haploid or diploid, depending upon the process involved in the initiation of embryo development.

From *a priori* considerations, the induction of parthenogenesis in the gymnosperms, especially by the use of foreign pollen, has always appeared to the writer as more likely of success than among the angiosperms where extensive stylar tissue is such a barrier. It is deemed relevant to here report the inadequacy of at least one *a priori* consideration. In none of the gymnosperms pollinated with a variety of foreign pollens over a four-year period, has there resulted a single case of parthenocarpy save in Ginkgo.

The Ginkgo concerned is a female tree growing in the Coker Arboretum at the University of North Carolina. There are no male trees in the arboretum, nor in the vicinity of the campus known to the author. The tree has never produced fruit save when pollinated with Ginkgo pollen in 1941.

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[1] This work was part of a program of Forest Genetics, supported by a grant from the General Education Board.

On April 7, 1943, pollen from *Cedrus deodara* (Roxb.) Loud, was liberally applied by broadcasting from near the top of the tree. The ovules were receptive as evidenced by the presence of a drop of mucilaginous exudate just outside the micropyle. As is well known, unpollinated ovules of Ginkgo soon die and drop from the branches. However, many ovules, after pollination with Cedrus pollen, did not abort as usual but persisted and slowly increased in size for several weeks before dropping. Only four seeds in all were recovered at the end of the season. Of the four, two were markedly reduced in size, averaging about half the size of normal seeds. One of these was sacrificed for examination and it appeared normal in every respect save size. What appeared to be an embryo was also observed. The three remaining seeds, after winter layering in sand, were planted in the greenhouse in early March. The remaining small seed promptly died, but the two larger seeds cracked their coats and appeared to be growing. However, after three months in the greenhouse, one seed died after emerging from the seed coat and swelling considerably, but without having put forth either radicle or shoot. The remaining seed is still alive and in precisely the same stage of development.

In the absence of adequate somatic tissue for a chromosome count, it is not known whether these embryos, if present, were haploid or diploid, and for the same reason it is impossible to say just what apomictic mechanism was responsible for their development, although pseudogamy is certainly indicated. In view of the genetic and morphological gaps between Ginkgo and Cedrus, it seems safe to say that syngamy was not involved.

The experiment was repeated this spring, but an unseasonably late frost killed all ovules and nearly denuded the tree of leaves.

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THE YELLOW-ORANGE ENDOSPERM OF MAIZE[1,2]

THE genetics of the yellow-orange endosperm color in maize has been examined by several investigators. A discussion of the

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[1] Preliminary note.

[2] Part of this work was done in the Genetics Department of the University of Missouri, on a fellowship of the Guggenheim Foundation.

pertinent literature will be given in another publication; suffice it to say here that two main genes with complementary effects, Y_1 and Y_3, are involved. These genes are located in chromosomes 6 and 2, respectively (Emerson, 1921; Perry and Sprague, 1936). Two other genes with effects similar to those of Y_1 have been reported: Y_2 in chromosome 5 (Eyster, 1931), and Y_4, linkage group unknown (Singleton).[3] Tests of Y_4 material received from Dr. Singleton indicate that Y_4 is identical with Y_1. The Y_2 stock is apparently lost. The location of this gene was made by examining its linkage with the gene vp_2 (vivipary or premature germination) (Eyster, 1931). Mangelsdorf (1926) described several genes for premature germination giving apparent linkage with yellow endosperm color, probably due to some physiological effect of premature germination on the pigmentation of the endosperm. It is possible that Y_2, like Y_4, is a synonym of Y_1.

The seed color in Y_1 Y_3 stocks may vary from a yellow similar in shade to that of a pale egg-yolk, to a deep orange found in South American maize and such strains of North American corn as Louisiana Yellow Creole Flint, which is probably of South American origin. As already reported by Andres (1939) and Sprague (personal communication), this variation is due to modifiers. The results obtained by us in crosses of South American deep orange strains with yellow North American ones are shown in Table 1.

Crosses of deep orange endosperm x white endosperm also show clearly the effects of modifiers on the Y_1 Y_3 basic condition. F_2 and F_3 ears and seeds show various shades of yellow and orange colors. However, by selection from this material it is, as shown in Table 2, possible to establish strains in which the difference orange *vs.* yellow behaves as if controlled by a single gene.

The location of this gene is as yet unknown. It will be examined in special experiments, but since the modifiers that may be present in tester stocks may obscure the segregation these experiments will take time.

A new gene complementary to Y_1, referred to below as Y_5, has been isolated from a cross of a deep orange Brazilian strain, "Cateto," with a tester strain y_1 y_3–*al* received from Argentina. The interaction of Y_1 and Y_5 produces a yellow endosperm color resembling that of a pale egg-yolk.

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[3] W. R. Singleton, in Maize Genetics Cooperation, *News Letter*, March 4, 1936. Cited with permission of the author.

TABLE 1

F_2 OF DEEP ORANGE ENDOSPERM STRAINS OF SOUTH AMERICA WITH YELLOW ENDOSPERM STRAINS OF NORTH AMERICA*

Pedigree (1942)	Deep orange		Orange		Yellow
832– 8	57	←	99	→	44
832– 1	←	144	→		89
832– 2			114	→	100
833– 3	←	60	→		71
833– 5			122	→	41
833– 1	←	146	→		
851– 6	58		48	→	72
851– 9	31	←	109	→	55
851– 3	18	←	84	→	29
851– 2	85	←	191	→	71
851–10	34	←	241	→	59

(1942) Pedigree	Deep orange	Orange	Orange-yellow	Yellow-orange	Yellow
882–10	16	59	70	31	24
882– 3	15	77 ←	..	→ 59	11

* The arrows indicate the presence of intermediate classes not possible to classify.

Data demonstrating the existence of this gene and its interrelations with Y_1 and Y_3 are presented in Table 3. Linkage with *Pl* and *al* indicates the presence of Y_1 and Y_3 in the deep orange strain used. The gene y_3 is identical with or very closely linked to *al* (Perry and Sprague, 1936). The apparent crossovers shown in Table 3 proved to be due either to hetero-fertilization or to classification difficulties. Cytology of chromosome 2 in a y_3–*al* plant showed no sign of deficiencies that could be responsible for both the white seeds and the albescent seedlings effects. Of course, these effects may be produced by a deficiency so small as to be invisible; however, the transmission of this condition both through ♀ and through ♂ gametes is normal.

The plants designated y_1 y_3–*al* were received from Argentina; they have been isolated from crosses of North American tester with South American deep orange endosperm strains. The Y_5 gene present in the y_1 y_3–*al* tester was presumably introduced from South American material. That Y_5 is indeed a gene for

TABLE 2

SIMPLE SEGREGATION FOR ORANGE VS. YELLOW IN A SELFED LINE

Pedigree (1942)	Orange	Yellow	Deviation 3 : 1	X^2	P
951–4	156	54	1.50	0.06	0.80
951–3	142	62	10.00	2.56	0.11
951–1	208	59	–7.75	1.19	0.29
Total	506	175	4.75	0.42	0.52

TABLE 3

INHERITANCE OF Y_5 AND INTERRELATIONS WITH Y_1 AND Y_3

I. F_2 Progeny 730–2 (1940). $\frac{Y_1\,pl}{y_1\,Pl}\ \frac{(Y_3\,Al)b}{(y_3\,al)B}\ \frac{Y_5}{Y_5}\ \frac{A}{a}$

Class	Genotypes	Seeds	Calculated 9 : 3 : 4	X²	P
orange-yellow	Y_1–Y_3–Y_5	226	220.03	0.162	0.68
yellow	Y_1–$y_3\,Y_5$	71	74.01	0.122	0.72
white	$y_1\,Y_3$–Y_5	98	98.68	0.004	0.95
white	$y_1\,y_3\,Y_5$				

	Seeds germinated		Plants obtained		A–B–Pl–	A-b Pl–	A–B–pl	A–b pl	a
	Al	al	Al	al					
orange-yellow	169	4	159	0	57	9	31	8	54
yellow	0	46	0	7	3	4	0	0	0
white	60	..	56	..	30	8	3	4	11
white	..	14	..	4	4	0	0	0	0

	Observed		Calculated on 28% crossing-over Y_1–Pl		X²		P	
	A Pl	A pl	A Pl	A pl	A Pl	A pl	A Pl	A–pl
orange-yellow	66	39	77.94	34.56	1.83	0.55	0.17	0.47
yellow	7	0	....	....	...	...	...	...
white	38	7	34.56	2.94	0.35	5.60	0.58	0.02
white	4	0	....	....	...	...	...	...

II. Backcross Progeny

730–9 × 770–7 (1940). $\frac{Y_1\,pl}{y_1\,Pl}\ \frac{(Y_3\,Al)b}{(y_3\,al)B}\ \frac{Y_5}{Y_5}\ \frac{A}{a} \times \frac{y_1\,pl}{y_1\,pl}\ \frac{(y_3\,al)B}{(y_3\,al)B}\ \frac{Y_5}{Y_5}\ \frac{A}{A}$

Class	Genotypes	Seeds	Calculated 1 : 1 : 2	X²	P
orange-yellow	Y_1–Y_3–Y_5	87	84.75	0.059	0.80
yellow	Y_1–$y_3\,Y_5$	81	84.75	0.166	0.69
white	$y_1\,Y_3$–Y_5	171	169.50	0.013	0.90
white	$y_1\,y_3\,Y_5$				

	Seeds germinated		Plants obtained		Pl	pl
	Al	al	Al	al		
orange-yellow	77	9	67	0	18	49
yellow	2	72	0	0	0	0
white	83	..	83	..	69	14
white	..	85	..	24	21	3

	Observed		Calculated on 28% crossing-over Y_1–Pl		X²		P	
	Pl	pl	Pl	pl	Pl	pl	Pl	pl
orange-yellow	18	49	21.0	54.0	0.43	0.46	0.52	0.50
yellow	0	0	...	...	...	...	...	...
white	69	14	54.0	21.0	4.16	2.33	0.03	0.13
white	21	3	...	...	...	...	...	...

endosperm color has been checked in the cross ♀ white endosperm $y_1\,y_1\,y_3\,y_3\,y_5\,y_5$ × ♂ yellow endosperm $Y_1\,y_1\,y_3\,y_3\,Y_5\,Y_5$, which gave about 50 per cent. of yellow and 50 per cent. of white seeds.

The new complementary gene gave, in extractions from a cross with Y_3, ratios of 36 yellow-orange: 9 yellow: 19 white ($Y_1 y_1 Y_3 y_3 Y_5 y_5$), and 9 yellow: 7 white ($Y_1 y_1 y_3 y_3 Y_5 y_5$). A ratio of 12 yellow-orange: 3 yellow: 1 white or 15 colored: 1 colorless ($Y_1 Y_1 Y_3 y_3 Y_5 y_5$), should also be expected. The relationships between the new gene and that producing similar ratios reported by Sprague[4] are not known.

The results obtained in back-crosses of $Y_1 y_1 Y_3 y_3 Y_5 Y_5$ to $Y_1 Y_1 Y_3 y_3 y_5 y_5$ are in accordance with expectations. Back-crosses of the same F_1 to $y_1 y_1 Y_3 Y_3 y_5 y_5$ gave the expected ratio 1 yellow-orange: 1 white when the F_1 was the ♀ parent. As ♂ parent on the $y_1 y_1 Y_3 Y_3 y_5 y_5$ tester the expected ratio 1 yellow-orange: 1 white was changed to approximately 1 yellow-orange: 3 white. Plants obtained from these seeds segregate normally according to the expectation on a $Y_1 Y_3$ basis and the F_1 pollen gave the expected 1:1 ratio on other $y_1 y_1 Y_3 Y_8 y_5 y_5$ stocks, indicating that we have to deal here with some kind of preferential segregation in favor of the y_1 gene in a particular ♀ genotype, similar to that found in pop-corn maize by Demerec (1929). This working hypothesis will be tested.

Deep orange endosperm strains crossed with several lines of white endosperm and with testers for all 10 chromosomes show segregation for only one yellow endosperm gene, Y_1 in chromosome 6. All testers and white endosperm strains crossed are $y_1 y_1 Y_3 Y_3$, and the interaction $Y_1 Y_5$ can be detected only in a $y_3 y_3$ genotype. The location of the Y_5 gene is therefore rather difficult, since all testers in our hands are Y_3 and unknown with respect to the Y_5 gene. The problem is difficult because it involves observing the segregation of three genes with cumulative effects on the endosperm color. Testers of $y_3 y_3$ constitution would simplify the matter, but unfortunately such plants grow very poorly (compare data in Table 3).

The deep orange endosperm strains of Brazil contain also a gene for yellow aleurone color located by proper tests in chromosome 7, and probably an allele of *Bn*$_1$ (Kavakan, 1924). The $Y_1 Y_3 Y_5$ genes and other modifiers of the $Y_1 Y_3$ control the carotenoid pigments, the color of the seeds varying from yellow to deep orange. The yellow aleurone gene, as reported already by Kvakan (1924) controls the yellow pigment only in the aleurone layer. These seeds are of a *lemon-yellow* color and are different from the yellow-orange seeds. The genetics of the yellow aleurone is independent from that of yellow endosperm, and the Bn_1 gene is also affected by modifiers. It is present in several strains of corn, making the analysis of the yellow-orange endosperm sometimes difficult.

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[4] G. F. Sprague, in Maize Genetics Cooperation, *News Letter*, March 6, 1938. Cited with permission of the author.

The carotenoid pigments in plants of different genotypes are being analyzed. The results so far obtained are in accordance with those of other authors (Mangelsdorf and Fraps, 1931; Randolph and Hand, 1940). Biological tests of the same material will be carried on in collaboration with the Physiological Department of the Medical School, University of Sao Paulo.

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EFFECTS OF X-RAYS ON HATCHABILITY AND ON CHROMOSOMES OF HABROBRACON EGGS TREATED IN FIRST MEIOTIC PROPHASE AND METAPHASE[1]

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IN 1904 Bergonié and Tribondeau found that in rat testes integumental cells were highly resistant while dividing cells were very sensitive to irradiation. In 1906 they concluded that x-rays affect the cell in direct proportion to (1) its reproductive activity, (2) earliness of its position in the series of divisions before maturity and (3) its degree of generalization in structure and function. In this publication they suggested that sensitivity of tumors to irradiation is due to the mitotic activity of their cells. In the same year Kraus and Ziegler found the time of organization of the equatorial plate the most sensitive stage and stated that it is less the kind of cell than its stage at time of treatment which determines sensitivity. Holthusen in 1921, after a thorough study of early cleavage in irradiated Ascaris eggs, agreed with the first and third conclusions of Bergonié and Tribondeau but substituted for the second the conclusion of Kraus and Ziegler. He observed that sensitivity is strikingly high when chromosomes begin to appear and is at its maximum at the construction of the metaphase plate.

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[1] This investigation has been aided by a grant (to P. W. Whiting) from the Rockefeller Foundation, for apparatus and technical assistance. The work was done at the Zoological Laboratory of the University of Pennsylvania and at the Marine Biological Laboratory, Woods Hole, Massachusetts. To these institutions the author is grateful for the use of laboratory facilities and of x-ray equipment. Valuable assistance was also given by the American Oncologic Hospital of Philadelphia through the use of x-ray equipment. Completion of the paper was aided by Swarthmore College.

Many investigators have studied the effect of x-rays on many kinds of cells since this earlier work and the majority, using diverse criteria, has favored the prophase as the most sensitive stage. In many cases, if not most, the metaphase has not been adequately studied either because of the difficulty involved in "catching" the cells in this relatively brief stage or of complication due to "physiological" effects, clumping and stickness of metaphase and anaphase chromosomes after irradiation.

A summary of more recent studies of irradiated oocytes is presented here. In the mature Drosophila female most of the older oocytes are in prophase stages in preparation for the first meiotic division and, with an occasional exception, none is further advanced than metaphase I. Patterson and Muller (1930) announced that both point mutations and chromosomal rearrangements were produced by raying either sex in Drosophila but in considerably greater abundance in the sperm than in the oocytes with a given dose and that the ratio of rearrangements to point mutations was higher in treated sperm than in similarly treated female germ cells. They postulated a difference connected with the sex of the cell as a factor in sensitivity. Patterson, Brewster and Winchester (1932) found that eggs retained in the ovaries of Drosophila became more sensitive to irradiation and concluded that the nearer eggs are to maturity, the more susceptible they are to x-rays with regard to production of chromosome breaks. This susceptibility, they suggested, is probably related to the condition of the chromatin.

Oliver (1934) showed that, when young Drosophila females are treated, sex-linked lethals, translocation and crossingover irregularities occur less frequently than with male treatment but that visible variations occur in equal numbers in both sexes. Shapiro and Volkova (1938) state that "Comparison of natural and induced mutation processes showed that latter occur more often in males than in females whereas former display no such difference, probably due to fact that x-rays induced more large chromosome aberrations in spermatoza.'' Glass (1939) found no translocations and fewer inversions in larvae from treated females than in larvae from similarly treated males in Drosophila while Sonnenblick (1940) showed for this species that ''especially susceptible to the radiations are the most mature oocytes.'' Schultz (1933) reported similar sex differences in response to x-rays in *Drosophila pseudoobscura.*

In 1938 the author stated that unlaid eggs of Habrobracon are most sensitive to irradiation in late metaphase I, almost equally so in latest prophase I (diakinesis) and least so in prophase stages before diakinesis. Adult (haploid male) survival from unfertilized eggs laid by treated females was used as the criterion. Later (1940) the data were presented in detail.

Metz and Boche (1939) observed that irradiation of adult females of Sciara gave rise to no gross chromosomal changes in F_1 larvae in contrast to irradiation of adult males and it was demonstrated by Metz and Bozeman (1940) that these eggs were in mid-prophase when treated. Reynolds (1941), by treating Sciara females restrained from ovipositing until eggs have reached stages later than mid-prophase, has found ''that oocytes increase in sensitivity as they pass through the late prophase stage of meiosis. . . . It is interesting to note that the metaphase is the most sensitive stage and that here even with comparatively low dosages a high frequency of breaks occurs.'' Reynolds concluded that difference in sensitivity is not a matter of sex but of chromosome condition at time of treatment, thereby agreeing with Kraus and Ziegler (1906). From new data (unpublished) Reynolds finds that early anaphase may be even more sensitive than metaphase.

The author, in 1941, announced that hatchability percentages of unfertilized Habrobracon eggs x-rayed in late prophase I with doses up to 400 r are significantly higher than those of controls and that lethal dose is about 35,000 r. Eggs treated in late metaphase I are sensitive to 50 r and lethal dose is about 1,250 r. There is no evidence of recovery in this stage. The dose-hatchability curves of the two stages differ, that of metaphase I showing a linear relationship to dose. In 1942 evidence against "physiological effect" as an important factor for metaphase I sensitivity was presented as well as preliminary cytological observations.

Bozeman (1942) reports that viable chromosome alterations are first detectable in Sciara larvae developing from eggs treated when chromosomes begin to shorten and move to metaphase I plate. Sensitivity increases through metaphase I and early anaphase I, then decreases during the arrested stage (anaphase). Just before fertilization sensitivity increases. A high frequency of intrachromosomal rearrangements and an unequal distribution of types of aberrations when different stages of meiosis are treated are reported and the latter fact is used as an indication that rearrangement is not delayed until fertilization.

MATERIAL AND METHODS

Two criteria for judging injury are used in the present study. One of these, hatchability, gives degree of lethal effect, both dominant and recessive, and is a very accurate measurement in Habrobracon where unfertilized haploid eggs are capable of development, where every egg can be accounted for, where the ideal environmental conditions are known and easily controlled and where there is available a stable wild-type stock consistently giving 96 per cent. hatchability or higher. The second criterion, chromosome changes in successive stages of the treated oocytes, is less satisfactory because Habrobracon chromosomes are too small (1 μ in diameter) and too numerous ($n = 10$) for very careful analysis. Giant salivary chromosomes are lacking. Enough has been learned from cytological observation, however, to make this method of some value in connection with hatchability data. The chromosome aberrations observed are necessarily gross ones and undoubtedly represent lethal effects in most cases, effects which escape observation in salivary chromosome studies.

Unmated mature Habrobracon females, when well fed on host caterpillars and then restrained from ovipositing for several hours by removal from host, store in each of their four egg sacs from two to five fully mature eggs which may be retained for at least thirty-six hours without injury. After a much longer period of storage they undergo resorption. They are all in late metaphase I with spindle attachment regions separated and about half way to the poles. The ends of the dyads are still in contact so that the chromosomes appear to be under tension. Occasionally, an egg in late diakinesis or in early metaphase I can be seen entering an egg sac. Successively younger prophase oocytes occupy the ovarioles anteriorly. Synapsis occurs as soon as youngest oocytes can be distinguished from oogonia. An ovariole is represented semidiagramatically in Fig. 1.

A count of eggs in dissected egg sacs of thirty females (1940) deprived of food for twelve hours gave the mean number in late metaphase I as 13.2 per female, of these plus those in early metaphase I and diakinesis as 14.3. In this earlier study eggs of treated (2,500 r) unmated females were collected in order of laying. The first few eggs laid failed to hatch and mean number of those laid before first to hatch was 13.1 per female, of eggs failing to produce adults before first adult was 14.5. It was concluded that all eggs in late metaphase had failed to hatch, that those in early metaphase and diakinesis had hatched but died as larvae. A pause in laying takes place after non-hatching eggs have been laid. At 30° C. this occurs between the sixth and seventh hour after the beginning of oviposition.

About sixteen oocytes which are unquestionably beyond the synaptic stage can be seen in each ovariole between the egg sac and the newly differentiated oocytes. With the technique used in this work control females lay

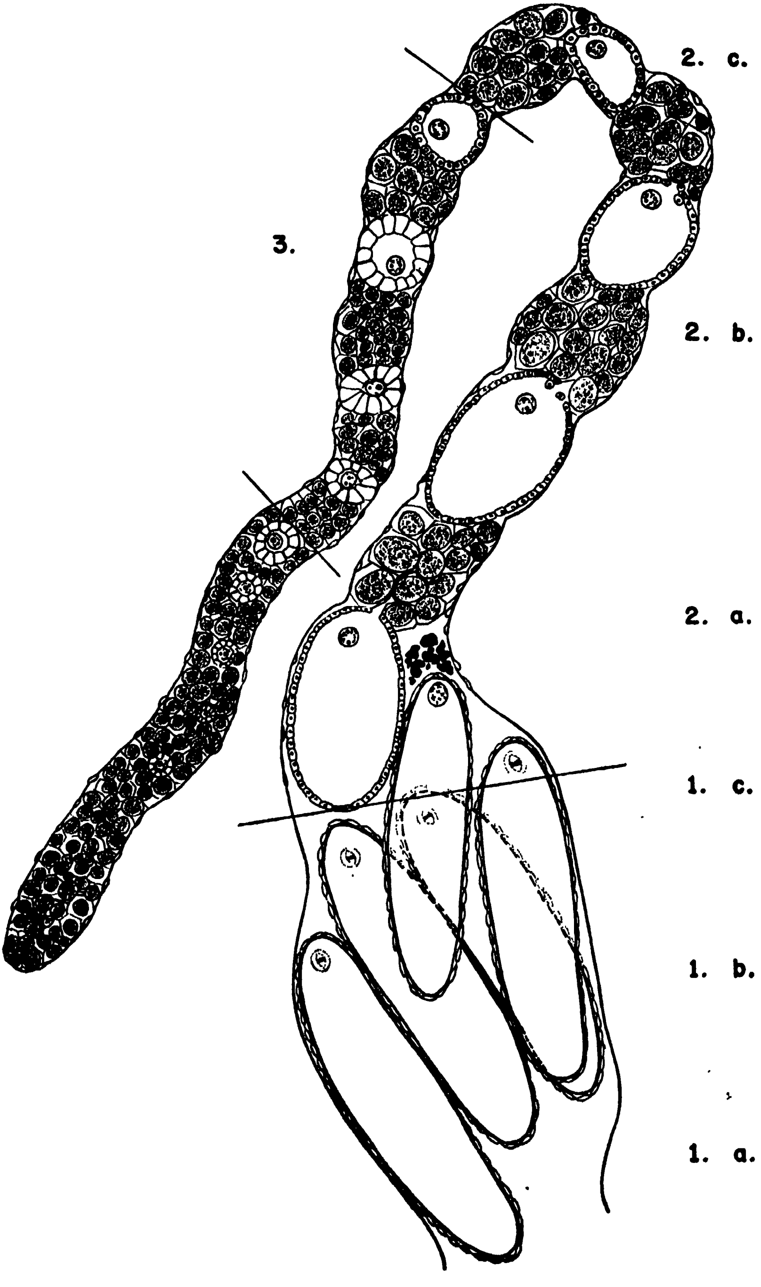

Fig. 1. A single ovariole (semidiagrammatic) showing regions used in classification of treated eggs. 1a, b and c include eggs in metaphase I stored in egg sac; 2a, b and c include eggs in metaphase I, late and mid prophase I; 3 includes eggs in early prophase I. All are post-synaptic. × 85.

an average of 11.3 (5,099/453) eggs per day which means that, at this rate, seven and one-half days would be required to lay the maximum number of metaphase I plus prophase I eggs present at any given time, that is, about eighty-five eggs. Since, in experiments, eggs are collected for one, two or three days only, they are without doubt post-synaptic when treated. Maximum number laid by treated females was twenty-one for day 1, forty-six for days 1 and 2 and 53 for days 1, 2 and 3. For those which remain to be laid or to disintegrate (if very young) there is, therefore, a margin of thirty-two or more before the post-synaptic number is exhausted.

The technique for experiments herein reported is as follows. Well-fed unmated females, all from the same vigorous wild-type stock, are deprived of food for twelve hours during which time, (as also throughout the experiments, except while being irradiated) they are kept at 30° C. unless otherwise stated. They are set immediately after treatment, still unmated unless otherwise indicated, on host caterpillars and are transferred to fresh hosts every two hours for six hours. Caterpillars, previously paralyzed by "nurse" wasps (females sterilized by x-rays), are used for this. The mean number of eggs per treated female during this six hour period has been 10.3 (13,835 eggs, 1,343 females), a number smaller than mean number of those observed to be in metaphase I in the egg sacs. At 1,800 r and all higher doses no egg laid during these first six hours after treatment has hatched among 8,991 studied, a clear indication that they were all in metaphase I when treated. Eggs are counted when laid and records are taken of their condition after a thirty-six hour incubation period. In later experiments hatchability has been substituted for adult survival since it is not affected by such environmental conditions as size of caterpillars, disease, etc.

When eggs which have been irradiated in prophase I are desired, females which have been laying for six hours as described above are removed from the host, placed in the incubator over night and then put on new hosts the day after treatment. During the first two hours of this second day of collecting, a small but variable number of eggs which were in metaphase I and diakinesis when treated are laid along with some which were in late prophase I. This variation is indicated by the great differences in hatchability of this group at any given dose. Females were transferred every two hours for six hours as on previous day. The process is repeated if eggs are to be collected on the second day after treatment. Treated females become practically sterile after this time, except when exposed to light doses.

Reference to Fig. 1 will help to make this method clear. For convenience in discussion all eggs laid during the day of treatment are said to belong to group 1a, b and c; those laid on the day after treatment to group 2a, b and c; those on the second day after treatment to group 3. All eggs in group 1 were in late metaphase I when treated, those in 2a in metaphase I, diakinesis or late prophase I, those in groups 2b and 2c probably in mid-prophase I and group 3 in early prophase I.

Two large control groups were studied during the course of the experiments. The first, in March, 1941, gave a hatchability percentage of 91.1 ± 0.6 (1,864/2,045), the second, in March, 1942, 96.6 ± 0.4 (1,522/1,575). This increase was the result of selection for high hatchability. In some experiments eggs of females were used as controls for eggs laid by the same females after treatment. This is legitimate since age of mother has no effect on hatchability for periods greatly in excess of any used. Standard errors are used throughout the paper.

Treatments were given at the Department of Radiology of the University of Pennsylvania, the Marine Biological Laboratory at Woods Hole and the American Oncologic Hospital of Philadelphia. At the University of Pennsylvania conditions of treatment were 200 kv., 20 ma., 15 cm. target distance, $\frac{1}{2}$ mm. copper plus 1 mm. aluminum filter, 500 r per minute. At Woods Hole the dual-tube selfrectifying outfit with a simultaneous cross-firing technique was used. The secondary voltage was 182 kv. and the tube current on each tube was 25 ma. The heavy glass of the tube walls and 5 mm. of bakelite of the tube shields gave the filtering value of 0.2 mm. copper shield. The output intensity was 560 r or 5,600 r per minute, depending on distance, 30 cm. for former, 9.5 for latter. At the American Oncologic Hospital conditions were 140 or 200 kv., 25 ma., and an inherent filter with the value of 6.0 mm. aluminum shield for 200 kv. and 3.8 for 140 kv. Output intensity varied from 220 to 1,350 r per minute. Time was the variable except where otherwise indicated. All high treatments for prophase studies were given at the Marine Biological Laboratory.

EXPERIMENTS

Eggs Irradiated in Late Metaphase I (1a, b, c). Dose-survival curve for Experiment 1 and dose-hatchability curves for Experiments 2–5, which concern eggs laid during six hours of the day of treatment, are plotted in Fig. 2. Data are given in Tables 1–3. Percentages are corrected since control survival and hatchability were

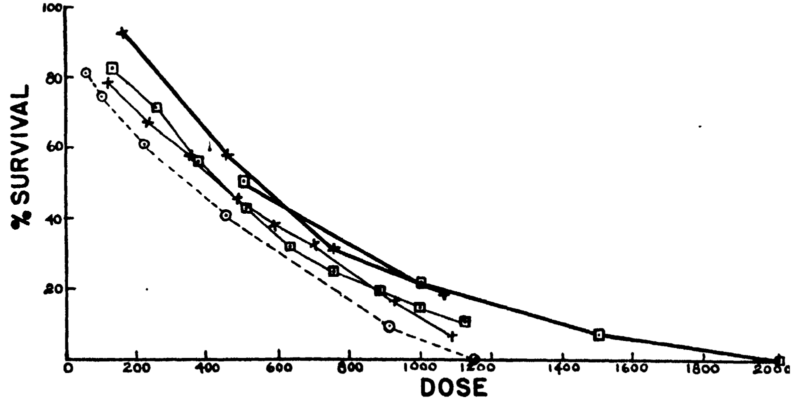

FIG. 2. The relation between x-ray dose in r units and (1) corrected adult survival percentages (dotted line) and (2) corrected hatchability percentages for treatments administered (a) at the University of Pennsylvania Hospital (light solid lines) and (b) at the American Oncologic Hospital (heavy solid lines). Eggs were treated in late metaphase I.

TABLE 1

CORRECTED ADULT SURVIVAL PERCENTAGES FOR EGGS TREATED IN LATE METAPHASE I; UNIVERSITY HOSPITAL. EXPERIMENT 1

Dose in r units	Number of eggs	Survival percentage
50	64	81.1
103	81	73.7
225	96	60.9
455	78	41.5
910	55	9.4
1,150	113	0.0

lower in earlier work. In subsequent figures and tables actual hatchability is given, control hatchability having become very high through selection. It is to be noted that the lower adult survival curve parallels the hatchability curves of the other experiments. The space between them represents larval and pupal mortality. Numbers are too small to have statistical significance in Experiments 1 and 2. Standard errors are computed for the others.

TABLE 2

CORRECTED HATCHABILITY PERCENTAGES FOR EGGS TREATED IN LATE METAPHASE I; UNIVERSITY HOSPITAL

Experiment 2			Experiment 3		
Dose in r units	Number of eggs	Hatchability percentage	Dose in r units	Number of eggs	Hatchability percentage
117	67	78.5	125	285	82.9 ± 2.8
233	55	66.9	250	305	71.3 ± 3.0
350	71	57.4	375	300	55.3 ± 3.2
484	94	45.5	500	260	42.7 ± 3.3
583	57	39.2	625	260	32.1 ± 3.1
700	120	32.1	750	434	25.6 ± 2.2
932	65	16.2	875	189	20.3 ± 3.1
1,089	39	6.1	1,000	136	14.5 ± 3.2
....	...	...	1,125	189	10.5 ± 2.3

Females used in Experiments 4 and 5 were x-rayed at

TABLE 3

CORRECTED HATCHABILITY PERCENTAGES FOR EGGS TREATED IN LATE METAPHASE I; ONCOLOGIC HOSPITAL

Experiment 4			Experiment 5		
Dose in r units	Number of eggs	Hatchability percentage	Dose in r units	Number of eggs	Hatchability percentage
159	262	92.1 ± 1.8	500	300	50.0 ± 3.0
450	465	57.3 ± 2.4	1,000	270	21.9 ± 2.6
750	697	31.1 ± 1.8	1,500	281	7.4 ± 1.6
1,060	307	18.2 ± 2.2	2,000	159	0.0

the Oncologic Hospital. A consistent difference exists in percentages of hatchability per given dose between their eggs and those of females irradiated at the University Hospital or at the Marine Laboratory. This may be due to a difference in method of calibration or in technique of handling.

Lethal dose for hatchability of eggs irradiated in late metaphase I is about 1,400 r for the University Hospital and Marine Laboratory treatments, about 2,000 r for the Oncologic Hospital. Fifty per cent. lethality is caused by 435 r and 500 r respectively.

Eggs Irradiated in Prophase I (2a, b, c, 3). An unexpected resistance to x-rays of late prophase I eggs was encountered in the first experiments reported (1938). Cytological study of the ovaries has demonstrated that all eggs classed as prophase have chromatin diffuse and but faintly stained by the Feulgen reaction, have completed synapsis and not yet reached diakinesis. This seems to justify the treatment of this group as a unit, at least for general conclusions.

Of nineteen experiments involving treatments of eggs in prophase I with doses ranging from 50 r to 750 r, seventeen had hatchability higher than controls, one the same as controls and one slightly lower. A summary of these nineteen experiments gives a hatchability of 95.2 ± 0.5 (1,569/1,647) with control hatchability of 91.1 ± 0.6 (1,864/2,045). The difference is 4.1 ± 0.8, five times its standard error. Further experiments are needed to check this suggestion, unorthodox at the moment, that small amounts of irradiation may stimulate development. Such stimulation, if it occurs, would have to be "physiological" in nature rather than chromosomal, perhaps comparable with that of freezing during diapause in certain insect eggs.

Uncorrected hatchability percentages of 2b plus 2c plotted against doses (Fig. 3) and data (Table 4) of Experiments 6, 7 and 8 illustrate the high resistance to x-rays of prophase I and the nature of the response. In

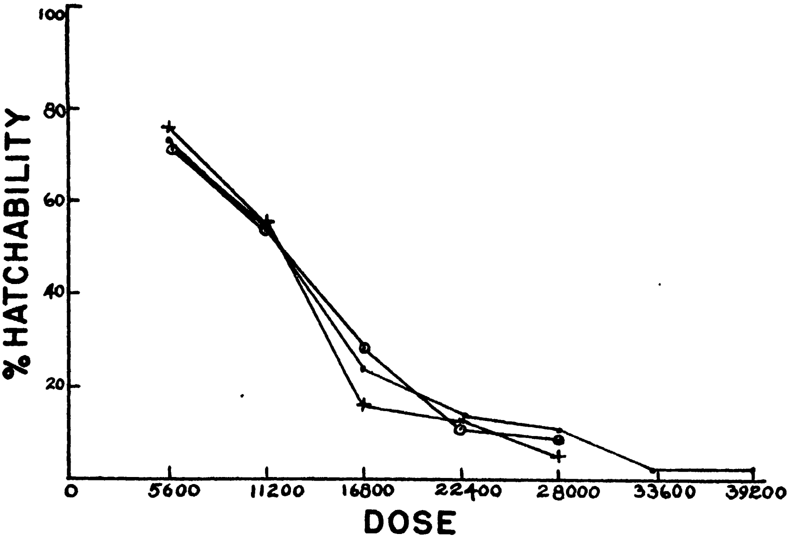

FIG. 3. Dose-hatchability curves of eggs treated in prophase I, three experiments.

Experiments 6 (1941) and 8 (1942) all females were irradiated at the same time for all doses and all were bred simultaneously. Experiment 7 (1942) was divided

TABLE 4

HATCHABILITY PERCENTAGES FOR EGGS TREATED IN PROPHASE I

Experiment	Dose in r units	2a		2b + 2c	
		Number of eggs	Hatchability percentage	Number of eggs	Hatchability percentage
6	5,600	51	7.8	98	75.7
	11,200	51	0.0	101	55.5
	16,800	52	0.0	101	16.0
	22,400	53	3.7	94	12.9
	28,000	48	0.0	119	4.9
7	5,600	173	43.9	393	72.5
	7,803	91	20.8	296	65.8
	11,200	227	40.5	259	56.7
	16,800	143	2.1	329	24.0
	22,400	242	3.3	264	13.6
	28,000	86	2.3	255	10.5
	33,600	224	0.4	295	2.0
	39,200	102	1.0	275	2.5
8	5,600	60	53.3	88	75.0
	11,200	25	12.0	111	58.5
	16,800	75	12.0	168	27.9
	22,400	65	9.2	187	11.6
	28,000	91	1.1	140	9.2
7 and 8	5,600	...	...	481	72.9 ± 2.0
	11,200	...	...	370	57.3 ± 2.6
	16,800	...	...	497	25.3 ± 1.9
	22,400	...	...	401	12.9 ± 1.6
	28,000	...	...	398	10.1 ± 1.5

into parts in order that larger numbers might be secured. Alternate doses were given at one time. After the highest treatments females appear unaffected and continue to live, sting hosts and feed although they lay almost no eggs beyond the second day after treatment since their ovaries have degenerated. 11,800 r produces 50 per cent. hatchability and 44,800 r approximates the lethal dose.

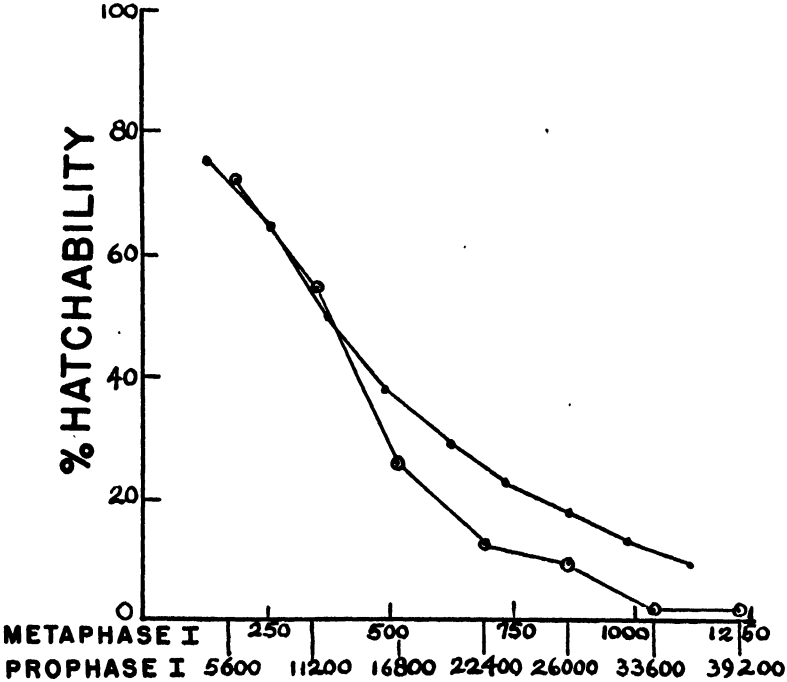

FIG. 4. Dose-hatchability curves of eggs treated in late metaphase I (●) and in prophase I (⊙) superimposed with points of 50 per cent. hatchability coinciding.

Although the chromosomes of the prophase I nuclei appear similar throughout the growth of the oocytes, hatchability data suggest that sensitivity to irradiation changes. A comparison has been made between hatchabilities of eggs laid during the second (2b) and third (2c) two hour periods of the day after treatment for each dose. 2a represents a mixture of eggs treated in late metaphase

I, diakinesis and latest prophase I and is, therefore, of no value in this study. Hatchability after treatment is higher in 2c (earlier) than in 2b (later) in eighteen of the twenty-one experiments. In five of the eight experiments where records were taken of hatchability on the second day after treatment (3, earliest prophase I studied) this group was found to have the lowest hatchability. In the other three experiments it fell between 2c and 2b in two and was higher than both in one.

These results indicate that hatchability of prophase I oocytes after irradiation decreases in the order of 2c, 2b and 3, *i.e.*, that earlier and later prophase I stages are more sensitive than the intermediate. This may be evidence for the dependence of sensitivity upon chromosome movement which would be greater in younger stages following synapsis and in older ones preparing for diakinesis.

Comparison of Eggs Treated in Late Metaphase I and in Prophase I. A comparison between the dose-hatchability curves of eggs treated in late metaphase I and those treated in prophase I is made in Fig. 4, where the curves are superimposed with points of 50 per cent. hatchability coinciding. Metaphase Experiment 3 is used here because of its large numbers and of the fact that it was done at the University of Pennsylvania so that hatchability is comparable with that following prophase treatments given at the Marine Laboratory. The two prophase I experiments (7 and 8) conducted within one week of each other are summarized for this comparison. Semilogarithmic representations of these curves are given in Fig. 5.

Eggs Irradiated and Fertilized by Untreated Spermatozoa. Are the lethal effects so far discussed dominant, recessive, or a mixture of both? If treated females are mated to untreated males two thirds of the eggs should be fertilized and, if any appreciable percentage of those that would fail to hatch as haploids could survive with the help of normal spermatozoa, it would be apparent that some of the effects induced in haploids are recessive. A preliminary test of the effect of such matings on metaphase I was reported in the earlier paper (1940). Three females were irradiated with 2,500 r and then mated. No effect was observed, but this test was not conclusive since 2,500 r is higher than the lethal dose.

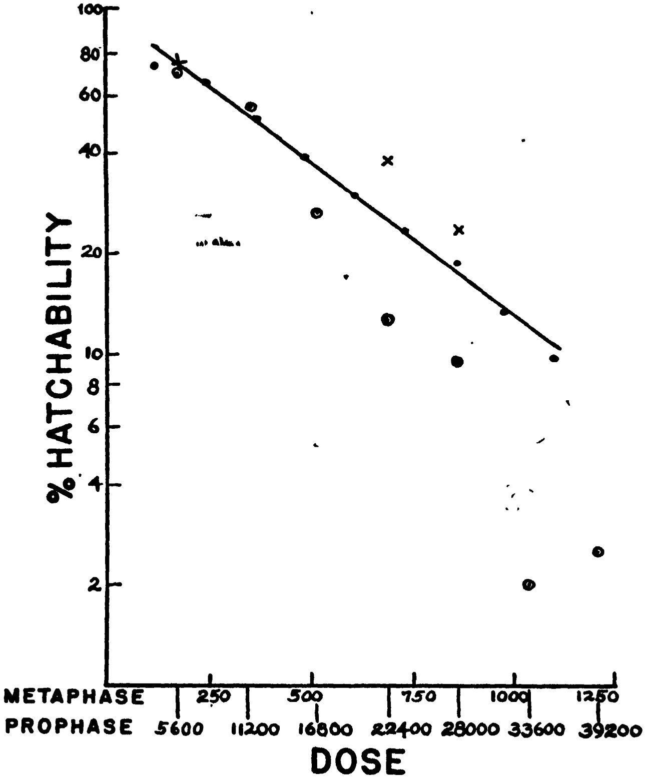

FIG. 5. Dose-hatchability curves, expressed semi-logarithmically, of eggs treated (1) in late metaphase I (●), (2) in prophase I, (a) continuous treatments (⊙) and (b) fractionated (×), superimposed with points of 50 per cent. hatchability coinciding.

In a recent experiment, summarized in Table 5, all the females of each dose group were treated and tested simultaneously. It will be seen from this summary that no apparent difference exists in hatchability between the mated and unmated groups in respect to eggs treated either in late metaphase I or in prophase I, at high and low doses. Certainly it can be concluded that most, if not all, of the lethal effects induced by x-rays in these stages are dominant.

Recovery. Four methods for the detection of recovery in this material may be considered. These are (1) comparison of hatchabilities of eggs laid immediately after treatment with those laid later; (2) withholding of females from host for intervals after treatment; (3) fractionation of treatments; (4) changes in time and intensity relationships of treatments. Objections to these methods arise from the facts that (1) not all eggs included in 2a, b and c are in exactly the same stage when treated and that this may also be true of 1a, b and c; (2) that almost all eggs have had time for recovery before being laid, if, as Sax (1942) and others believe, recovery takes place within an hour after treatment if at all; (3) that changes within the cell may take place between treatments or during long treatments so that the total dose is not given to any cell at the same stage. However, the data which have been accumulated are herewith presented for what they are worth.

TABLE 5

HATCHABILITY PERCENTAGES FOR EGGS OF TREATED FEMALES MATED TO UNTREATED MALES

Stage treated	Dose in r units	Unmated females		Mated females	
		Number of eggs	Hatchability percentage	Number of eggs	Hatchability percentage
Metaphase I (1a + 1b + 1c)	560	319	39.8 ± 2.7	318	40.5 ± 2.7
Prophase I (2b + 2c)	5,600	137	71.5 ± 2.6	126	70.6 ± 4.0
	22,400	100	19.0 ± 3.9	182	19.2 ± 2.9
Controls	0	127	98.4 ± 1.1	363	98.6 ± 0.6

Of twenty-seven experiments in which metaphase I hatchability was studied, with doses ranging from 116 r to 1,500 r, percentages of hatchability were higher in 1b than in 1a for 12, lower for 15; higher in 1c than in 1b for 20, lower for 7; higher in 1c than in 1a for 16, lower for 11. No difference exists between 1a and 1b, but 1c tends to have slightly higher hatchability than 1a or 1b. This presents no valid argument for recovery.

Females were treated and kept for varying lengths of time after treatment before being allowed to oviposit. No delay was great enough to affect hatchability of controls. It is believed that the majority of eggs remain relatively static until laying begins in females under experimental conditions since they have been away from hosts and at 30° C. for twelve hours before treatment and have had ample time in which to store up all metaphase eggs permitted by space or the food supply. Results for eggs treated in metaphase I are summarized in Table 6. Differences are in no case significant and there is no evidence that recovery occurs or that eggs treated in prophase I have entered the egg sacs during the period between treatment and oviposition for hatchability of 1c eggs would be noticeably increased by such a phenomenon. Metaphase I eggs retained for twenty-four hours before being laid have the same length of time for recovery as prophase I eggs in the regular experiments so that time for recovery plays no important part in differential sensitivity.

TABLE 6

TESTS FOR RECOVERY OF EGGS TREATED IN METAPHASE I

Dose in r units	Number of eggs	Interval for recovery in hours	Hatchability percentage
200	167	0	83.1 ± 2.9
200	214	5	87.0 ± 2.3
484	94	0	38.3 ± 5.0
484	93	24	32.3 ± 4.8
500	119	0	38.6 ± 4.4
500	163	4	44.8 ± 3.9
750	196	0	17.8 ± 2.7
750	162	2	17.3 ± 2.9

Data for effects of fractionation on metaphase I are not extensive since but one experiment was carried out to test it. Twelve females were exposed to 700 r and placed on hosts at once. Twelve others were exposed twice to 350 r with a twenty-four hour interval between. Hatchability of 1a, b and c eggs laid by the first was 26.6 ± 4.0, of the second 26.3 ± 5.2. There is no significant difference.

A time-intensity experiment was conducted at the Oncologic Hospital (Table 7, metaphase I). There are no evidences of any effect of changing time-intensity relationships. Conditions of treatment may have been unsuitable either in size or degree of difference to give evidence of recovery.

The conclusions reached from these experiments are (1) that position in the egg sac appears to have little or no relation to sensitivity and (2) that there is no evidence for recovery of eggs treated in late metaphase I.

Because of the uncertainty as to numbers of chromosome stages among prophase I eggs and as to whether these stages remain relatively stable in females after they have stored eggs for twelve hours (although all evidence indicates that they do), the question of recovery in relation to eggs treated in prophase I becomes a more difficult one to attack. Experiments were nevertheless conducted because of the possibility that the effect of fractionation might be greater than could be explained by egg movement from a less to more sensitive stage of vice versa during the intervals between treatments.

TABLE 7

COMPARISON OF HATCHABILITY PERCENTAGES FOR EGGS TREATED WITH DIFFERENT INTENSITIES

Stage treated	Dose in r units	Number of eggs	r/m	Time	Hatchability percentage			
Metaphase I (2a + 2b + 2c)	485	182	212	137 s.	53.8 ± 3.6			
	485	119	375	77 s.	56.2 ± 4.6			
	750	230	220	205 s.	30.0 ± 8.0			
	750	245	1,130	40 s.	26.5 ± 2.8			
					2a	2b	2c	2b + 2c
Prophase I	8,661	560	84.9	102 m.	46.4	69.3	80.1	73.8 ± 2.4
	8,661	544	84.9	102 m.	55.9	69.4	61.1	70.3 ± 2.6
	7,803	387	5,600	84 s.	20.8	65.3	67.5	65.8 ± 2.7
	11,200	351	76.5	146 m.	40.6	59.7	62.3	60.4 ± 3.3
	11,200	578	5,600	2 m.	20.3	57.8	64.5	61.0 ± 2.8
	11,200	486	5,600	2 m.	40.5	52.4	68.0	56.7 ± 3.1

Females receiving continuous and fractionated treatments for each dose were bred at the same time. Two-hour intervals were allowed between fractional doses and all groups were kept from oviposition for an hour after the end of treatment so that each irradiated egg would have at least one hour in which to recover. During intervals between treatments and between final treatment and first oviposition, females were kept at low room temperature in order to retard maturing of eggs and consequent chromosome movement. Females receiving fractionated treatments had four additional hours after the initial treatment in which to permit prophase changes, if such occur. Temperature was kept at 32° C. during oviposition, in contrast to the 30° C. usually employed. Further conditions of treatment and hatchability percentages are presented in Table 8.

TABLE 8

TESTS FOR EFFECTS OF FRACTIONATION OF DOSE ON HATCHABILITY PERCENTAGES FOR EGGS TREATED IN PROPHASE I

Dose in r units	Number of eggs	Time in minutes	Hatchability percentage				
			2a	2b	2c	3	2b + 2c
5,600	360	10	58.3	71.1 ± 4.9	72.2	69.7	71.5 ± 3.8
5,600	606	3–3–4	48.6	74.1 ± 4.0	70.7	67.8	72.9 ± 3.3
22,400	161	4	25.0	15.2 ± 5.2	21.1	8.2	17.8 ± 4.1
22,400	271	1½–1½–1	13.2	36.9 ± 5.9	18.5	12.8	31.5 ± 4.7
22,400	262	4	21.5	18.9 ± 5.3	19.1	4.5	19.0 ± 3.8
22,400	1,131	1½–1½–1	11.8	38.5 ± 3.2	22.4	11.9	35.6 ± 2.8
28,000	452	5	2.3	9.6 ± 2.0	14.0	7.2	10.5 ± 1.9
28,000	581	2–1½–1½	8.1	23.2 ± 4.1	15.3	3.4	19.9 ± 3.0

The difference in hatchability of 2b plus 2c between continuous (71.5 per cent.) and fractionated (72.9 per cent.) for 5,600 r is not significant. For 22,400 r fractionated, hatchability is 13.7 per cent. higher in one experiment (31.5 per cent. – 17.8 per cent.) and 16.6 per cent. higher in the other (35.6 per cent. – 19.0 per cent.). For 28,000 r fractionated, hatchability is 9.4 per cent. higher (19.9 per cent. – 10.5 per cent.). These differences are not great but if the three prophase groups are studied individually, it will be seen that, in fractionated, hatchability is lower for 2a, much higher for 2b and about the same for 2c. The first can be explained by the higher number of eggs laid on day 1 by females receiving continuous treatment. The other two suggest strongly a differential response to fractionation by different stages of prophase I. Data of day 3 show no consistent differences and are not considered in detail since much oocyte degeneration is going on at this time, especially after the higher doses, and results are not so strictly comparable.

For 22,400 r differences in 2b are 21.7 ± 7.9 and 19.6 ± 6.3; combined, the difference is 21.0 ± 4.8. For 28,000 r the difference is 13.6 ± 4.7. The highest previous hatchability after continuous treatment in 2b for 22,400 r is 15.8 per cent. and the highest in any stage for this dose is 21.0 per cent.; for 28,000 r in 2b it is 9.6 per cent., in any stage 14 per cent.

In view of the fact that there is no evidence for egg movement, as pointed out above, and that, after fractionation, hatchability of 2b is significantly higher than that of the group most resistant to continuous treatment with the same dose, it can be concluded that fractionation has had an effect.

The 5,600 r treatment is not conclusive. There is a difference in hatchability in 2b but it is not significant and earlier experiments (continuous) have given a higher hatchability (76.0) in 2b than this one with fractionation. The difference may be so slight at this lower dose and under the conditions of treatment as to require many experiments to prove it, or there may be no effect. A curve based on hatchability of 2b with fractionated exposures is plotted in Figure 5 where it will be observed that it suggests a straight line; *i.e.*, that a "mixed" curve has been changed by fractionation to a one-hit curve.

Time-intensity variations in treatment are difficult to obtain where such high doses as those necessary for prophase I effects are required. In fact, accurate continuous high doses are impossible to get in most institutions. Experiments here reported (Table 7) give inconclusive results.

From fractionation tests it might be expected that 2b would show the effects of time-intensity differences if recovery is the basis for both. Hatchability of 2b for 7,803 r (high intensity) is lower than for 8,661 r (low intensity) in spite of lower dose. For 11,200 r 2b is likewise lower for the higher intensity-shorter time group but in neither case is the difference significant.

Egg-laying "Patterns." It is obvious that, if any environmental factor such as high dose, mating, etc., should alter the egg-laying "pattern," that is, the percentages of total eggs per female which are laid on each day of collection, or the total number itself, the conclusions herein reported would become invalid. Data have been exhaustively analyzed from this angle but a detailed discussion of them would be out of place here. They may be summarized as follows.

Mean number of total eggs laid on days 1 plus 2 is constant for all experimental conditions used, 18.9–23.5. Mode for each experiment is 24. This constancy appears to be due to the limited and constant food supply, less than could be utilized by the females under any of the experimental conditions. Percentage laid on day 1 plus 2a, $\frac{(1+2a)}{\text{Total}}$, is a constant, as are 2b and 2c. Percentage laid on day 1 varies between experiments but never includes any eggs treated in prophase I. When it is high, 2a is low and vice versa. When percentage laid in 2a is low its hatchability is high, indicating that fewer eggs treated in metaphase I have been retained until day 2. This variability in 2a makes it of no use in this study. Since some eggs almost always hatch in 2a after doses lethal to metaphase I but not to prophase I, 2b plus 2c contains practically none of the former and so this group has been used in estimating prophase I resistance.

Stages at Which Death Occurs with "Lethal" Doses. The persistence with which the Habrobracon egg continues meiosis after irradiation is to be noted. All irradiated eggs which are well enough fixed and stained to show chromatin at all, show a continuation of the meiotic phenomena or an attempt at such continuation after being laid. This is true even after a dose of 200,000 r.

It is difficult to be certain about a comparison of "lethal" doses for eggs treated in each stage. Long and exhaustive tests would be required to find them exactly. When eggs treated in metaphase I with a dose of 2,016 r and in prophase I with a dose of 44,800 r are incubated for twenty-four hours, fixed and stained, all are seen to have undergone some development and all appear to have died. Of the metaphase I eggs (2,016 r), 2.4 per cent. die in the first cleavage, 7.1 per cent. with a few nuclei, 71.4 per cent. with many nuclei and 19.0 per cent. at blastoderm stage. Of prophase I eggs (44,800 r) 1.1 per cent. die at first cleavage, 30.4 per cent. with a few nuclei, 54.3 per cent. with many nuclei and 14.1 per cent. at blastoderm.

In spite of their relative sensitivity as indicated by lethal dose, eggs treated in metaphase I will continue development after very heavy treatments. All developed to the stage expected (early cleavage) after 4,550 r, some to sixteen nuclear stage (expected) after 15,000 r, to metaphase II after 25,000 r, to pronucleus after 35,000 r and one to anaphase II after 200,000 r. At doses of 4,550 r and lower there is no apparent slowing up of development. At 15,000 r some appear to develop at normal rate (with ten minutes variation). No records of time were kept at the higher doses and no observations were made on prophase I with doses higher than 44,800 r.

CYTOLOGY

A summary of the cytological effects is given in this paper in order that use may be made of all available information in the discussion. A detailed account with drawings is in preparation. The extremely small size of the chromosomes and their relatively large number should be kept in mind by the reader who tends to be critical of the limited observations. About 2,500 eggs were prepared and studied.

Technique. Eggs from control and treated females were incubated according to control schedules, dropped into fixative (formalin-acetic-alcohol), punctured at the posterior end to facilitate fixation, treated with the

Feulgen nucleal-reaction and mounted whole in balsam. Whole mounts were made of ovaries with comparable technique. Control hatchability tests were made of eggs treated at the same time as those fixed.

Meiosis in Controls. The majority of students of hymenopteran cytology would question the statement that the stored oocyte is in metaphase I. They state that the chromatin has reverted to a resting stage, or has formed an abortive maturation spindle or a compact clump or a composite body, etc. Speicher (1936) states that the most advanced eggs in the Habrobracon egg sac are in ''early anaphase of the first maturation'' and ''further progress in maturation is stopped until after oviposition.'' The author prefers to call this late metaphase of the first maturation and has checked repeatedly the observation of Speicher that distinct chromosomes are present, are in the form of tetrads and are ten in number. The conclusion must be drawn that Habrobracon differs from many other Hymenoptera in having meiosis uninterrupted between metaphase I and anaphase I by an ''amorphous'' or ''compact'' clump of chromatin, or that its chromosomes, in spite of their small size, must retain more easily their individuality when fixed.

The meiotic stages as described by Speicher will be outlined briefly. For several minutes after being laid the maturation spindle appears much as in the unlaid egg except that it has been moved from the dorsal to ventral side of the egg during oviposition. It then rotates to a position vertical to the egg surface and passes into telophase I. The second division follows immediately. The four groups of chromosomes (1a, 1b, 2a, 2b) lie in a row roughly perpendicular to the egg surface. During anaphase II polar nuclei 1a and 2a remain stationary, 1b moves close to 2a, and 2b (ootid nucleus) sinks deeper into the egg, a nuclear membrane forming as it moves. Nucleus 1a quickly disintegrates, 1b and 2a unite, form a metaphase plate, which divides and disintegrates.

Meiosis in Eggs Irradiated in Metaphase I. Chromosomes in eggs just laid show no effects of irradiation.

The dyads are distinct and appear to be uninjured. Effects of treatment are first visible in anaphase I when fragments can be seen within the spindle and between the chromosome groups. These are often almost as large as the chromosomes and usually appear to be double. They are considered terminal deletions. No evidence of a bridge has been seen in any of the large number of eggs studied. In subsequent meiotic stages these fragments can still be seen and counted. In division II, fragments, small and apparently single, may be observed as well as bridges or both.

The number of fragments in division I varies with dose as does the number of eggs with fragments (Table 9).

TABLE 9

COMPARISON OF HATCHABILITY PERCENTAGES AND OF CYTOLOGICAL OBSERVATIONS FOR EGGS TREATED IN METAPHASE I

Dose in r units	Hatchability percentage	Percentage of eggs without deletions	Hatchability percentage estimated	Deletions per egg	Power
500	50.0	37.0	62.9	1.0	...
1,000	21.0	15.3	38.4	1.92	0.94
1,500	7.1	6.1	24.2	2.39	0.53
2,000	0.0	4.2	17.0	2.84	0.58

In Figure 6 are plotted the dose-hatchability curve for eggs irradiated at the same time as those fixed and the curve for the percentages of eggs without deletions. It is apparent that some eggs with deletions must hatch. If there is but one deletion in an egg and it is double and terminal, the ootid nucleus would have a 1:1 chance of receiving an unbroken chromatid since the injured dyad may go to either pole in division I and there is no bridge in this division to prevent a broken chromatid from entering the ootid nucleus (Beadle and Sturtevant, 1935). If there are two broken dyads the ootid nucleus would have one chance in four of being free from injury. This will explain discrepancies between these two curves in Fig. 6 and furnish a reason for their approaching each other at high doses when most eggs have several deletions.

The effects of 500 r and of 1,000 r vary as the 0.94 power of dose. As dose increases this power becomes smaller (Table 9). This can be explained by supposing either that deletions become smaller as they become more numerous and can not be seen so readily or that when a deletion is once free from the attached portion of the chromosome further injuries in it undergo restitution. The latter has been tested recently by Sax (1942) who finds that effective breaks are much more frequent in the centric chromosomes than in acentric fragments.

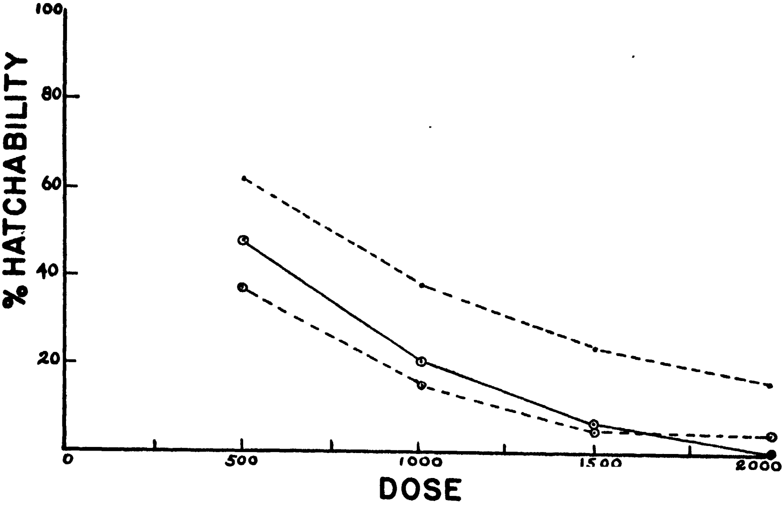

FIG. 6. The relation between x-ray dose in r units and (1) hatchability percentages (solid line), (2) percentages of eggs without fragments in telophase I (lower dotted line) and (3) percentages of eggs containing no fragments plus ½ containing one and ¼ containing two, a "theoretical" hatchability curve (upper dotted line). Eggs were treated in late metaphase I.

Bridges in division II are found more frequently between nuclei 2a and 2b (ootid nucleus) than between 1a and 1b. Since nucleus 1a disintegrates very soon after it is formed, the attached monad might return to 1b when freed in the disintegrating nucleus and bridges in the outer spindle would be temporary. The ootid nucleus forms a wall as it moves toward the center of the egg. When a bridge is present this nucleus becomes shaped like a tear drop and then spherical with a small knob at one side, connected by the bridge to 2a. The ductility of the bridge and its resistance to breakage is remarkable.

Meiosis in Eggs Irradiated in Prophase I. Eggs irradiated in prophase I show the same evidence of injury as those just described except that bridges may be formed in division I. These are not frequent enough, however, to explain high resistance of this stage (Beadle and Sturtevant, 1935). No detailed statistical analysis of injuries to chromosomes treated in prophase I has been made as yet. At low doses (500 r to 2,000 r) 3.4 per cent. of the eggs have shown injury. Only one of these was a bridge in division I. At 15,000 r (30 per cent. hatchability) 36.4 per cent. show injury. In this group as well as that treated with 42,000 r (almost lethal) the same types of injury occur. When a bridge is present in division I it is often, if not always, permanent and when nucleus 1b moves toward 2a in division II the bridge can be seen as a loop bulging at the side and connecting nuclei 1b and 2a.

Cleavage in Irradiated Eggs. Bridges have been found in cleavage I in eggs treated in both stages indicating that, if chromatids were already split when treated, there had occurred lateral fusion of broken ends. If they were not split when treated the split must have been "incomplete" when it occurred in the broken chromatid after treatment. Bridges and fragments appear frequently in cleavage and the latter are formed by two breaks in a bridge which release a thickened mid-region of the bridge. Such fragments are pointed at each end in contrast to those observed in meiotic divisions.

DISCUSSION

Muller (1940) states that "eggs and young embryos tolerate a much lower dose than sperm, yet they do not have as many genetic changes induced in them." If, by toleration of lower dose, is meant lower lethal dose and, by genetic changes, a term broad enough to include dominant lethal effects, neither part of the statement holds for

Habrobracon. Dominant lethal effects are induced in all eggs treated in metaphase I at about 1,400 r, in spermatozoa at 10,000 r and in eggs treated in prophase I at 45,000 r. Spermatozoa are not actually inactivated by doses much larger than 10,000 r but neither are eggs by doses much larger than lethal since they continue meiotic phenomena under such conditions. These facts strongly support Reynolds (1941) in his conclusion that the sex of the cell has nothing to do with its response to x-rays but rather that the condition of the chromatin determines cell sensitivity.

The extreme and unexpected sensitivity of metaphase I in Habrobracon oocytes has caused some investigators to suggest that the effect is "physiological" and not due to direct chromosome change. The attention of the author was called to the work of Lamy and Muller (1939) who show that diploid Drosophila embryos are no more sensitive than triploid at the same x-ray doses, an argument against chromosome change and, therefore, in favor of "physiological" effects. The author (Whiting and Bostian, 1931) found that haploid (male) larvae are more sensitive to irradiation than diploid (female) larvae of Habrobracon, as expected if chromosome change is responsible. These are normal forms in this species in contrast to the anomalous triploids in Drosophila.

Sax and Swanson (1941) describe the clumping and sticking together of metaphase chromosomes in Tradescantia microspores after irradiation due to "physiological" effect. Such an effect, if produced in metaphase I oocytes of Habrobracon, must be very transitory. Eggs treated in this stage and in prophase I all have chromosomes and fragments, when present, distinct in outline in stages following oviposition and no fusion bridges have been seen. Absence of a low dose threshold for injury and absence of a high one for clumping and retardation of meiosis and early cleavage for doses up to 15,000 r furnish additional evidence against any serious "physiological" effect in this stage. Since both metaphase I and prophase I eggs, after their respective lethal doses, present the same pattern in regard to degree of development before death, causes of death would appear to be of the same nature for each.

The fact that the lethals induced in these oocytes are dominant has been used as an argument for "physiological" injury. This is inconsistent with the view held by all investigators in this field, that dominant lethal effects in spermatozoa of Drosophila are chromosomal.

The linear proportionality between dose and hatchability as well as the other facts about metaphase I oöcytes outlined above can be explained by the following hypothesis. At the time of treatment, dyads are under tension since spindle fiber regions have started toward the poles while ends are still held in contact by chiasmata. When a hit breaks a pair of chromatids (a dyad) proximal to a chiasma the parts will separate, too far to rejoin but not far enough for the fracture to be seen. Subsequent complete terminalization and separation of dyads in anaphase I leave fragments and there are no bridges. A broken dyad produces a bridge in division II in either spindle with equal frequency. If a single chromatid is broken the fragment remains attached to its uninjured partner and appears in division II (McClintock, 1938). An egg with a single deletion will fail to hatch if unfertilized, and, even if fertilized, when the deletion is large. If two chromatids in a "lug" (distal to chiasma) were broken and the chromatids fused laterally, an occasional bridge in division I might be expected. Tension is much less in this region so that restitution usually occurs when a hit causes a break in this short length of chromosome.

As explained above, an egg with one double fragment has one chance in two of hatching, an egg with two such fragments, one chance in four (if the possibility of two breaks in one dyad be left out of consideration). A "hatchability" curve can be made by adding to the eggs showing no fragments, one half of those showing one, and one fourth of those showing two. Such a curve (Figure 9) is considerably higher than the actual hatchability curve of treated controls but parallel to it. Probably if all deletions could be seen and the theoretical hatchability curve constructed, it would coincide with the actual curve, or lie very close to it.

These data conform with the conclusions of Sax (1938), Belgovsky (1939), Marshak (1939), Faberge (1940), Carlson (1941), and Pontecorvo (1941) in respect to one-hit phenomena but appear to differ from those of Newcombe (1942).

Sax (1940) found that a single hit usually does not break sister chromatids in or after late prophase and Muller (1940) is not convinced that double breakage from a single ionization has been proved in any form. Delbrück (1940) questions the production of two breaks by a single ionization on physical grounds. In Habrobracon, bridges in division II following treatment in late metaphase I can not be explained by chromosome splitting following breakage in view of the structure of the metaphase tetrad. It therefore seems probable that a single hit has broken two chromatids in late metaphase I.

The prophase I hatchability curve (Figs. 3, 4, 5) does not show a linear relation to dose. Rather, it resembles the dominant lethal curve for Drosophila sperm as described by Fano and Demerec (1940). "The production of dominant lethals is apparently proportional to the x-ray dose for small dose and the survival ratio decreases exponentially for high dose. On the whole, however, the 'dose-action' curve does not seem to fit the 'single hit' type very well. This would indicate that the phenomenon is not simple, which is very plausible." More than one stage is included in prophase I and undoubtedly aberrations of different kinds are induced.

When hatchability of prophase I exposed to fractionated treatments is plotted semilogarithmically (Fig. 5) an approximate straight line is formed and the modified curve stands in contrast to that of high continuous doses where a speeding up effect appears. Sax (1942) has found that fractionated dose decreases the frequency of complex aberrations. He attributes this to restitution between exposures. The elimination of a large proportion of complex rearrangements might change a "mixed" curve into a one-hit curve. The variation in hatchabilities at dose 16,800 r, the point near which the trend of the curve seems to change, may indicate the neighborhood of a threshold at which a new phenomenon enters the picture. Doses above and below this point were chosen for fractionation tests with the idea that lower dose might not respond to fractionation (one-hit part of curve), that high dose might do so (mixed part). Results do not contradict this. If Newcombe's (1942) suggested delayed restitution at high doses begins to take effect in the neighborhood of 16,800 r for prophase, and repeated smaller doses have no cumulative effect of this type, an increase in hatchability with fractionated treatments would be expected only at higher doses.

The period of high sensitivity of prophase I, late prophase (latest prophase I can not be estimated because of mixture with metaphase I eggs) is the same as that which responds to fractionation. The higher sensitivity might be explained by supposing that terminalization, beginning in this stage, would increase breakage because of increased tensions. An explanation for a relationship between terminalization and fractionation effects seems difficult to formulate. A detailed discussion of the cytological basis for prophase I response to x-rays would be of little value without further study of the chromosomes. Obviously, broken ends of sister chromatids fuse, if bridges in division II are an indication of this. It is suggested that, although ionizations may injure these prophase I chromosomes as frequently as they do the later more condensed ones, the injuries are temporary and restitution is high because of the small amount of movement, and therefore of tension, at time of treatment. They ultimately undergo all the movements and tensions of those treated in metaphase I and also additional ones connected with their condensation and arrangement on the metaphase plate but they emerge unmodified, or with non-lethal changes, unless treated with very heavy doses.

It is known that many deficiencies are viable in the heterozygous condition, lethality being dependent on gene content of the segment lost. Since diploid larvae are more resistant to irradiation than haploid, recessive deficiencies must be induced in somatic cells. It is surprising, in view of these facts, that all the lethal effects of irradiation herein described appear to be dominant. If breaks proximal to the chiasmata are the ones which tend to be permanent, these would necessarily result in large (and therefore dominant) terminal deletions. Deletions small enough to act as recessives might not have a lethal effect on the haploid embryo until after hatching and therefore would not be detected with methods used in the present work. In the limited tests made with fertilized eggs it is also possible that a few deficiency heterozygotes surviving would not noticeably affect hatchability ratios.

Attempted explanations of differential sensitivity to x-rays may be classified into those that involve differences (1) in original breaking of a chromosome strand (*true* difference in sensitivity), (2) in number of strands broken per ionization and (3) in restitution.

Marshak (1937) advanced the first of these and Darlington (1942) and Sax and Swanson (1941) have since suggested that high nucleic acid content, as in metaphase chromosomes, would prevent breakage. This argument can not apply to results herein reported.

Sax (1940) suggests the second, that, as number of strands in a chromosome increases, breaks per ionization will increase and (Sax and Swanson, 1941), as chromatids become more widely separated, one only will be broken by an ionization. In the tetrad breakage of a chromatid with two strands would not differ in effect from breakage of one with a single strand since the chromatid acts as a unit until time of first cleavage. In both prophase I and metaphase I lateral sister chromatid fusion takes place, and, presumably, breakage of two chromatids by one hit.

Factors which might inhibit restitution in one stage and not in another are (a) lateral sister chromatid fusion (Sax and Swanson, 1941), (b) movement of chromosomes (Metz and Bozeman, 1940; Sax, 1940; Wilson and Nebel, 1939; Muller, 1940), (c) tension (as distinct from movement), (Goodspeed, 1929; Stone, 1933; Mather, 1934; Sax and Swanson, 1941), (d) time difference between treatment and division (Stone, 1933; Lea, 1938), (e) retardation factor late in cycle (Newcombe, 1942).

In respect to Habrobracon eggs (a) there is lateral sister strand fusion in both stages; (b) chromosomes in more resistant stage probably move more than in sensitive; (c) tension appears greater in sensitive stage; (d) all eggs treated in prophase I and most of those treated in metaphase I have more than one hour for recovery and when each stage is given identical long periods for recovery there is no evidence of effect; (e) if twenty-four hours are allowed for restitution in some metaphase I eggs while others with same treatment are laid at once, restitution still does not take place in former, lateral sister chromatid fusion has taken place in both. If Newcombe's factor slowing up restitution late in cycle exists, it does not prevent relatively rapid lateral sister chromatid fusion and so can not be due to any inabilty of injured ends of chromatids to undergo fusion.

It must be concluded that tension and its influence upon restitution is the most convincing explanation for the results herein reported. With this must be included a relatively rapid recovery in chromosomes treated in prophase I. That the degree of tension is due to conditions peculiar to the first meiotic division seems probable.

SUMMARY

A. Unlaid Habrobracon eggs x-rayed in late metaphase I and allowed to develop parthenogenetically

(1) Show a linear decline in hatchability from 50 r to 1,400 r, approximately the lethal dose.

(2) Show no change in hatchability percentages with aging after treatment, time-intensity differences or fractionation of dose.

(3) Continue meiosis and early cleavage after doses several times lethal, at rates not noticeably different from controls.

(4) Show fragments but no bridges in division I, bridges, fragments or both in division II, fragments and bridges in cleavage.

(5) Give a curve expressing dose-percentages of eggs without fragments which at high doses converges toward the dose-hatchability curve.

(6) Show a number of fragments per treated egg which varies as the 0.94 power of dose between 500 r and 1,000 r, 0.53 power between 1,000 r and 1,500 r, and 0.58 power between 1,500 r and 2,000 r.

B. Unlaid eggs x-rayed in prophase I and allowed to develop parthenogenetically

(1) Give hatchability percentages equal to or higher than controls from 50 r to 750 r.

(2) Show a decline in hatchability from 1,000 r to 44,800 r, approximately the lethal dose.

(3) Give a hatchability curve which appears to be linear at low doses and to become "mixed" at high doses.

(4) Show no significant change in hatchability in preliminary test of time-intensity differences but an increase with fractionation of dose.

(5) Continue meiosis and early cleavage at lethal dose at rates not noticeably different from controls.

(6) Show fragments and bridges in division I, either or both in division II, fragments and bridges in cleavage.

C. Unlaid eggs treated in either stage

(1) Show no change in hatchability when fertilized by untreated spermatozoa.

(2) Develop as far as blastoderm in about twenty per cent. at approximately lethal doses.

D. It can be concluded that

(1) Consistent and correlated cytological and hatchability patterns of eggs treated in late metaphase I appear

to prove conclusively that death is due to chromosome breaks arising from single hits.

(2) Consistent differences in cytological effects and in nature of dose-hatchability curves correlated with consistent differences in stages of chromosomes at time of treatment argue strongly for the dependence of ionization effects upon the physical (or chemical) condition of the chromosomes.

(3) High sensitivity of metaphase I, the stage in which tensions of chromosomes have been considered very high, argues strongly for the dependence of ionization effects upon the tension of chromosomes. Other suggestions made to explain differential sensitivity of chromosomes in different stages do not apply to material used in this study.

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CHROMOSOME STUDIES ON EIGHT SPECIES OF SCIARA (DIPTERA) WITH SPECIAL REFERENCE TO CHROMOSOME CHANGES OF EVOLUTIONARY SIGNIFICANCE. II

(*Continued from page 121*)

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Sciara species VII

1. SOURCE AND GENERAL CHARACTERISTICS

This species was collected in each of two greenhouses within five miles of each other. The laboratory stock was derived from the offspring of two females collected at the same time in a greenhouse located in Newtown Square, Pa. Individual mated females of this species produce offspring of predominantly one sex.

2. SOMATIC AND GONIAL METAPHASE CHROMOSOMES

The somatic chromosome group (Fig. 1b) is composed of three pairs of rod-shaped chromosomes and one pair of V-shaped chromosomes. The gonial metaphase group (Fig. 1a), on comparison, shows four pairs of rods, one pair of V's. Of these chromosomes, two rods are without homologues in the somatic complex, and are considered to be the "limited" chromosomes.

3. SALIVARY GLAND CHROMOSOMES

The salivary gland chromosomes of this species are very difficult to spread and stain. Various modifications of the technique used for the other species were tried without success. Slides of more than eight hundred larvae were made in order to obtain four good preparations and a dozen fair ones.

A great deal of asynapsis and subsequent twisting of the two homologues about each other made the accurate determination of band pattern very difficult. The question whether asynapsis of the two homologues is always accompanied by a band pattern difference can be answered in the negative according to the evidence presented by this species. A short region of the C chromosome (Fig. 25b) is not synapsed and shows no pattern difference. Likewise, in the two short regions shown in Fig. 28b the banding patterns are the same. These cases do not rule out the possibility of submicroscopic differences which may be responsible for the asynapsis.

Small differences between homologues. The arrow in Fig. 25a (end 1 of the C chromosome) points to a vesicular band in one homologue which is apposed to a nonvesicular band in the other homologue. This kind of small difference was also found in another species to be mentioned later. End 2 of the C chromosome shows no irregularities (Fig. 25c). End 2 of the A chromosome (Fig. 28b) shows a split end and a terminal deficiency marked by an arrow, in addition to three short asynaptic regions in which no pattern difference can be detected.

"Repeats." There were no "repeats" observed in this species. The X chromosome, which has shown "repeats" in other species, is regular (Fig. 26).

Rearrangements. A short intercalary inversion of at least three bands was found in end 1 of the B chromosome (Fig. 27a). A similar inversion was found in end 1 of the A chromosome (Fig. 28a). These two inversion figures have been diagrammed in Fig. 27b. The asynapsis in end 1 of the A chromosome (Fig. 28a) does not seem to be due entirely to the inversion, but partly to a lack of band homology. As has been shown above, the C chromosome of the laboratory stock did not carry any inversions. But on examination of the slides made from the second collection of this species, the C chromosome was found to be heterozygous for the subterminal inversion shown in Fig. 25e. This collection was made in a greenhouse at Media, Pa. The entire figure of this inversion is diagrammed in Fig. 25d.

Sciara nacta Johannsen

1. SOURCE AND GENERAL CHARACTERISTICS

This very small species seems to be widely distributed in greenhouses, having been found on numerous occasions as far north as Springfield, Mass., and as far south as Tampa, Fla. The laboratory stock was derived from the offspring of a female collected at Gradyville, Pa.

Mated individual *S. nacta* females produce bisexual progenies. The species thrives well in the laboratory and may be kept in mass cultures without difficulty.

2. SOMATIC AND GONIAL METAPHASE CHROMOSOMES

The somatic metaphase chromosome group is composed of four pairs of rod-shaped chromosomes. Fig. 26 shows a somatic group with the homologues of three pairs separated. The gonial metaphase group (Fig. 2a) shows four pairs (homologues separated) of chromosomes homologous with those of the soma. In addition, there are two large unpaired, rod-shaped chromosomes. These are the "limited" chromosomes. This is the third species in which there has been found two unpaired rod-shaped "limited" chromosomes. The possible significance of this fact will be treated in the discussion.

3. SALIVARY GLAND CHROMOSOMES

Although the individuals of this species are small, the salivary gland chromosomes are very favorable for study. They smear and stain readily, giving, for the most part, sharply banded figures. The chromosome ends are easily differentiated.

Fig. 29a shows end 1 of the C chromosome. Fig. 29b shows end 2 of the C chromosome with its characteristic "puff." Figs. 31a and 31b are the two ends of the A chromosome.

Small differences between homologues. Only one consistent small difference was seen in this species. Fig. 30a (end 1 of the B chromosome) shows a small difference, indicated by the arrow, in which a single band in one homologue is apposed to a thick band in the other homologue. End 2 of the B chromosome is regular (Fig. 30b).

"Repeats." End 1 of the X chromosome (Fig. 32a) is characterized by a bifurcated tip and a subterminal knotting of the chromosome approximating a figure eight. This latter condition is due to a double "repeat" in which the synapsis of three linearly separated homologous regions has occurred. No cells were found in which the band pattern of these three regions was clear. The

banding pattern of end 2 of the X chromosome is seen in Fig. 32b.

Rearrangements. No rearrangements were found in the laboratory stock from which the above data were compiled. However, two short inversions were found, one in each of two other stocks. Fig. 29c shows one of these, which is a short subterminal inversion in end 1 of the C chromosome. The other is a short subterminal intercalary inversion of end 1 of the C chromosome (Fig. 29d). This figure also shows (see arrow) a terminal deletion in which one homologue is minus its terminal band.

Sciara fenestralis Zett., form II

1. SOURCE AND GENERAL CHARACTERISTICS

This species was found in a number of greenhouses within a fifty-mile radius of Philadelphia and also in a meadow in Ridley Park, Pa. Individual mated females of this species produce unisexual progenies in which occasional individuals of the opposite sex are found. The laboratory stock was derived from the mating of male and female offspring of two females captured in a greenhouse at Newtown Square, Pa.

2. SOMATIC AND GONIAL METAPHASE CHROMOSOMES

The somatic metaphase group (Fig. 7b) consists of two pairs of V-shaped chromosomes and two pairs of rod-shaped chromosomes. In addition to those chromosomes which are homologous to the somatic metaphase chromosomes, the gonial metaphase group has one large V-shaped "limited" chromosome (Fig. 7a).

3. SALIVARY GLAND CHROMOSOMES

The salivary gland chromosomes of this species offer no technical difficulties, but they twist about each other and have a high degree of asynapsis, making it difficult to be sure of band pattern. The X chromosome is the only one of the complex which shows no irregularties. Ends 1 and 2 of the X are seen in Figs. 35a and 35b, respectively.

Small differences between homologues. The A chromosome is exceptionally long and twisted. End 1 is shown in Fig. 33a. End 2 (Fig. 33c) has a short subterminal asynaptic region (see arrow) in which an optically homogeneous region in one homologue is apposed to a banded region in the other. Fig. 33b, a short central region of the A, shows a single band apposed to a vesicular band. Two other small differences were found in the inverted C chromosome. One of these, marked by the arrow in Figure 36a, shows a thin band in one homologue apposed to a thick band in the other. The second is seen opposite the arrow in Fig. 36b. In this case, a pair of thin bands is apposed to a large vesicular band.

"Repeats." No "repeats" were found in this species.

Rearrangements. Fig. 33d shows a short intercalary inversion of the A chromosome. This is the same kind as that diagrammed in Fig. 27b. The B chromosome (Fig. 34) is heterozygous for a subterminal inversion of about two thirds of its length. Within this long inversion, there is a short inversion as shown in the figure. The C chromosome (Fig. 36b) is heterozygous for a nonterminal inversion involving about three fourths of the chromosome. Fig. 36a shows the banding pattern of the synapsed ends of the C chromosome which are not shown in Fig. 36b.

Sciara fenestralis Zett., form I

1. SOURCE AND GENERAL CHARACTERISTICS

This material was collected in a greenhouse at Colmar, Pa. The laboratory stock was derived from the offspring of a single female, since the species produces bisexual progenies. The sex ratio in this species always favored the males. The longer the stock was kept in the laboratory the greater the preponderance of males. In the eighth month after collection, the females cast only males and the stock was lost. This species is closely related to *S. fenestralis,* form II, as stated above. Although mating reactions were observed between the two species, no offspring were ever produced from reciprocal matings.

2. SOMATIC AND GONIAL METAPHASE CHROMOSOMES

The somatic metaphase chromosomes (Fig. 4b) consist of two pairs of V-shaped chromosomes and two pairs of rod-shaped chromosomes. The gonial metaphase group (Fig. 4a) has two large V-shaped chromosomes which have no homologues in the soma. These are the "limited" chromosomes.

3. SALIVARY GLAND CHROMOSOMES

Although the somatic metaphase chromosome groups of *S. fenestralis,* forms I and II, are very similar, the banding pattern of the chromosome ends of the two species is different (figures on plate V). In the material studied, the salivary gland chromosomes of form I show fewer aberrations than those of form II, although form I has a "repeat" in the X chromosome which form II lacks.

Figs. 40a and 40b show ends 1 and 2 of the B chromosome; these show no asymmetry.

Small differences between homologues. End 1 of the A chromosome (Fig. 39a) shows no aberrations, but end 2 of the B chromosome (Fig. 39b) shows a small difference marked by the arrow. In this case, a thin band is apposed to a thick one. Another small difference is seen opposite the arrow in the synapsed ends of the C chromosome (Fig. 38b). In this latter case, there is a thin band in one homologue which has no counterpart in the other.

"Repeats." The X chromosome possesses a "repeat" of at least three bands causing it to loop back on itself when the two homologous regions synapse (Fig. 37b). However, it is difficult to be sure that only three bands are repeated. Fig. 37a shows the pattern of end 1 of the X which does not show clearly in figure 37b. Fig. 37c shows end 2 of the X chromosome.

Rearrangements. Fig. 38a shows a subterminal inversion involving about five sixths of the chromosome. Fig. 38b shows the band pattern of the short free ends which do not show in Fig. 38a.

DISCUSSION

1. GONIAL AND SOMATIC GROUPS

Since the genus *Drosophila* and the genus *Sciara* are included in the order Diptera, but widely separated, and since they can be studied by comparable methods, it is important to investigate any significant differences between them in chromosome organization, in the hope that some light may be shed on the evolutionary processes at work. For the most part, the writer can only point out in this paper certain similarities and differences. The full significance of the data will not be clear until further studies can be made on *Sciara*.

It is of interest to note that all the species of *Sciara* thus far examined have four pairs of chromosomes in the soma. In addition to the chromosome groups presented in this paper, those of *S. coprophila, S. similans, S. pauciseta* and *S. prolifica* were published by Metz (1926a, 1926b), and those of *S. reynoldsi* and *S. ocellaris* were published by Crouse (1939). Mr. Carson is preparing for publication figures of *S. impatiens.* Hence, the chromosome complexes of a total of fourteen species have been examined, all of which show four somatic pairs of chromosomes.

The forms of these four pairs, however, differ and fall into three groups as pointed out above. Of the fourteen species, two have all rod-shaped chromosomes, four have three rod-shaped pairs and one V-shaped pair, and eight have two rod-shaped and two V-shaped pairs. There is at the present time no indication which, if any, of the above groups is the ancestral type. One species in each of the three groups is widely distributed; namely, *S. ocellaris,* two V's—two rods; *S. impatiens,* one V—three rods; *S. nacta,* four rods. The method of change from V to rod by shift of the spindle fiber locus and *vice versa* has been postulated and discussed by Crouse (1939).

Evolutionary changes in the "limited" chromosomes have been discussed by Metz (1938b). It seems desirable to summarize at this time the occurrence and nature of these bodies in the fourteen species studied to date.

Three of the fourteen species have no "limited" chromosomes. Metz postulates that these probably represent the more recent species. Of the remaining eleven species, three have been found to have two rod-shaped "limited" chromosomes each, and eight have been found to have V-shaped "limited" chromosomes. Metz (*op. cit.*) has discussed the fact that those species possessing "limited" chromosomes may have more than one V-shaped "limited" chromosome in some individuals but never less than one. Hence, the assumption is that the "limited" chromosomes in those species possessing them do have some necessary function.

The numbers of the species representing the three different conditions with respect to "limited" chromosomes suggest that the V-shaped "limited" chromosome is the ancestral type. The presence of three species without "limited" chromosomes which are otherwise the same in chromosome number and general behavior leads to the supposition that the "limited" chromosomes are gradually being lost from this genus. Additional evidence on the form and number of "limited" chromosomes is presented here from three species, each of which has two rod-shaped "limited" chromosomes. In each of these cases, the rod-shaped "limited" chromosomes appear to be morphologically identical. Such a condition could conceivably arise by a fragmentation through the apex of an equal armed V-shaped chromosome.

Since those species found to have rod-shaped "limited" chromosomes always have two of them, the question arises concerning the genetic activity of each. If these rods do represent halved V-shaped "limited" chromosomes, then it seems probable that the genetic activity of the V-shaped chromosome is distributed on either side of its apex or centromere. If this were not so, one would expect to find some races or species possessing a single, short rod-shaped "limited" chromosome. Observations on members of three different races of *Sciara species* XXIII showed no single rod-shaped "limited" chromosomes. Further investigation is needed to determine (1) whether species exist in nature with only one rod-shaped "limited" chromosome, (2) whether those species possessing two rod-shaped "limited" chromosomes could survive with only one.

2. SALIVARY GLAND CHROMOSOME CONSIDERATIONS

Rearrangements. Five of the eight species reported in this paper show chromosome rearrangements similar to those found in *Drosophila* (for a review of this subject see Muller, 1939) and *Chironomus* (Bauer, 1936). In addition to these, three other species, making a total of eight out of fourteen species, show gross chromosome rearrangements. Hence, this genus can not be said to differ fundamentally in this respect from *Drosophila* or *Chironomus.*

The species for which no rearrangements have been recorded are certainly not to be considered different in this respect on the basis of the evidence on hand. This point is illustrated by the fact that each of two different collections of *S. ocellaris* in Delaware County, Pa., yielded a large inversion, whereas previous collections had not done so.

The preliminary observations on the *S. ocellaris-reynoldsi* species hybrids, like the *Chironomus* hybrids reported by Goldschmidt, 1942, showed no typical rearrangement figures (Metz and Lawrence, 1938). This led to the thought that possibly such changes did not occur in *Sciara.* However, the work of Dr. Pauline Rohm (unpublished) analyzing and mapping the *S. ocellaris-reynoldsi* hybrid C chromosome has shown that the two homologues differ by two inversions.

"Repeats." The subject of "repeats," their method of origin and their probable significance has been discussed by Bridges (1935). The "repeats" on which Bridges based his discussion were found in *Drosophila melanogaster* autosomes. Metz and Lawrence (1938) published photomicrographs of "repeats" as seen in *Sciara ocellaris* and in *S. ocellaris-reynoldsi* hybrids. These "repeats" were in the X chromosomes. The "repeats" found in six of the eight species reported in this paper have all been present in the X chromosomes. The

significance of this phenomenon has not yet been discovered.

According to the postulates of Bridges, these ''repeats'' probably arise through duplication and then subsequent translocation of the duplicated region within the chromosome. The ''repeats'' studied in this paper would tend to bear out this theory, for the sizes of the loops in ''single repeats'' vary from a tight loop in which the repeated regions are adjacent, Fig. 10a, to loose loops of different sizes, Figs. 13a, 24b and 37b. A similar situation obtains in the ''double repeats,'' as shown by Figs. 19b and 32a. The banding pattern of the repeated regions is difficult to obtain clearly, and thus far the writer has not been able to demonstrate any similarity of pattern among the repeated regions of the several species studied.

Small differences. The nature and frequency of occurrence of small differences in the banding pattern of five of the eight species studied here are different from those recorded for *Drosophila.* Hence, there is reason to believe that these represent a phenomenon which is characteristic of the genus *Sciara.* The differences between homologues pointed out above in the observations can be classified into five categories; namely: (1) ''single'' band deficiencies, (2) vesicular band apposed to disc-shaped band, (3) thick band apposed to thin, (4) homogeneous region apposed to banded, (5) terminal band deficiency.

The first, third and fifth of these categories have been discussed for *Sciara ocellaris* and the findings compared with those of other investigators by Metz (1937, 1938a). The second category is one not found in *Drosophila,* but was represented in this material by two cases. The third category was most numerous, being represented by four cases. Possibly these cases are ones in which the thick bands represent a duplication of material, thus providing for chromosome enlargement. The fourth category was found in several cells from different individuals of one species. That a real difference exists here between the homologues is indicated by the asynapsis. The fifth category was found consistently in individuals of only one race of one species. This type of deficiency was described by Kikkawa (1938) for *Drosophila ananassae.* Kikkawa was of the opinion that in his material these apparent terminal deletions were not deletions but represented additions on one homologue. He found no subterminal cases of this or any other type of small difference in his material and concluded that none existed.

Taxonomy. The taxonomy of the genus *Sciara* is at the present time in a confused state. There exists considerable overlapping between American and European species, and, indeed, there exists some discrepancy between the existing key to the species and the description of some of the species therein (Johannsen, 1917). In order that at some later time names may be given to those species which I have referred to by number and that those which I have called by name may be checked, I am including a photomicrograph of the right wing of the male of each species (Plate VI) and an outline drawing, drawn with the aid of a camera lucida, of the gross morphology of the hypopygium of the male of each species as seen from the ventral surface (Plate VII).

Pinned specimens and alcoholic specimens of each of the species have been deposited with Dr. Alan Stone at the U. S. National Museum, Washington, D. C.

SUMMARY

1. Eight different species of *Sciara* have been captured in the wild and reared in the laboratory.

2. Somatic chromosome complexes of all eight species show four pairs of chromosomes, which fall according to shape into three categories: (a) All rods, two species; (b) Three pairs of rods—one pair of V's, two species; (c) Two pairs of rods—two pairs of V's, four species.

3. Gonial metaphase figures show four species to possess V-shaped "limited" chromosomes, three species to possess rod-shaped "limited" chromosomes and one species to possess no "limited" chromosomes.

4. Salivary gland chromosome studies disclose the following: (a) Six of the eight species have "repeats" in

the X chromosome, of which four are single and two are double; (b) Five of the eight species show chromosome inversions of varying complexities and lengths; (c) Six of the eight species show "small differences" between homologues in the salivary gland chromosome banding which are seemingly characteristic of the genus.

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EXPLANATION OF FIGURES, PLATE IV

25–28 *Sciara species VII.* Salivary gland chromosomes.

25a End 1 of ''C'' chromosome. Arrow indicates a vesicular band whose homologue is nonvesicular.

25b Intercalary region of ''C'' chromosome in which there is asynapsis with no visible band difference.

25c End 2 of ''C'' chromosome.

25d Diagrammatic representation of the manner of pairing of the inverted homologues of 25e.

25e Subterminal inversion of ''C'' chromosome found in different stock from that from which 25a, b, and c drawings were made.

26 ''X'' chromosome.

27a End 1 of ''B'' chromosome which shows a small inversion.

27b Diagrammatic representation of inversion shown in 27a and 28a.

27c End 2 of ''B'' chromosome.

28a End 1 of ''A'' chromosome. Great deal of asynapsis is due to inversion which makes each homologue loop at right angles to its longitudinal axis, as well as to band differences.

28b End 2 of ''A'' chromosome. Asynapsis shown here in some regions without band difference. Arrow points to a terminal difference in which one homologue is deficient for a small amount of material.

29–32 *Sciara nacta.* Salivary gland chromosomes.

29a End 1 of ''C'' chromosome.

29b End 2 of ''C'' chromosome with characteristic terminal puff.

29c Short subterminal inversion of ''C'' chromosome in a second stock of *S. nacta.*

29d Short subterminal inversion of ''C'' chromosome with vesicular band terminal deletion, marked by the arrow, found in a third stock of *S. nacta.*

30a End 1 of ''B'' chromosome. Arrow indicates a small band difference wherein one homologue contains a compound band and the other homologue at the corresponding locus only a single band.

30b End 2 of the ''B'' chromosome.

31a End 1 of the ''A'' chromosome.

31b End 2 of the ''A'' chromosome.

32a End 1 of the ''X'' chromosome showing the characteristic bifurcated tip and the knotted loose figure eight caused by the synapsis of three apparently homologous regions. (Double repeat).

32b End 2 of the ''X'' chromosome.

EXPLANATION OF FIGURES, PLATE V

33–36 *Sciara fenestralis,* form II. Salivary gland chromosomes.

33a End 1 of ''A'' chromosome.

33b Short intercalary piece of ''A'' chromosome in which arrow points to solid band apposed to a vesicle.

33c End 2 of "A" chromosome. Arrow indicates a short homogeneous region opposite a banded region.

33d Short intercalary inversion of "A" chromosome.

34 Inverted "B" chromosome with short included inversion.

35a End 1 of "X" chromosome.

35b End 2 of "X" chromosome.

36a Synapsed ends of inverted "C" chromosome. Arrow indicates thin band apposed to thick band.

36b Large "C" chromosome inversion. Arrow indicates a vesicle apposed to a double band.

37–40 *Sciara fenestralis*, form I. Salivary gland chromosomes.

37a Duplicate of end of "X" chromosome, shown beneath it, in which banding pattern is better.

37b Repeat loop of "X" chromosome. Repeated bands synapsed to form loop.

37c Other end of "X" chromosome.

38a Long subterminal inversion of "C" chromosome.

38b A different view of the ends shown in 38a in which banding pattern of the two non-synapsed ends is good.

39a End 1 of "A" chromosome.

39b End 2 of "A" chromosome. Arrow points to "small difference" in which a thin band is apposed to a thick band.

40a End 1 of "B" chromosome.

40b End 2 of "B" chromosome.

EXPLANATION OF FIGURES, PLATE VI

41–44, 46–48 Photographed at magnification indicated above Figure 1. All photographs were made from the dorsal surface of the right wing of the male.

41 *Sciara nacta.*

42 *Sciara agraria.*

43 *Sciara species XXV.*

44 *Sciara fenestralis*, form II.

45 *Sciara prolifica* (Felt), Johannsen's var. b. Magnification scale indicated beneath it.

46 *Sciara species XXIII.*

47 *Sciara species VII.*

48 *Sciara fenestralis*, form I.

EXPLANATION OF FIGURES, PLATE VII

Magnification scale drawn beneath each figure represents .1 mm. Each figure represents the ventral surface of a male hypopygium.

49 *Sciara species VII.*

50 *Sciara nacta.*

51 *Sciara prolifica* (Felt), Johannsen's var. b.

52 *Sciara species XXV.*

53 *Sciara fenestralis*, form II.

54 *Sciara fenestralis*, form I.

55 *Sciara species XXIII.*

56 *Sciara agraria.*

PLATE IV

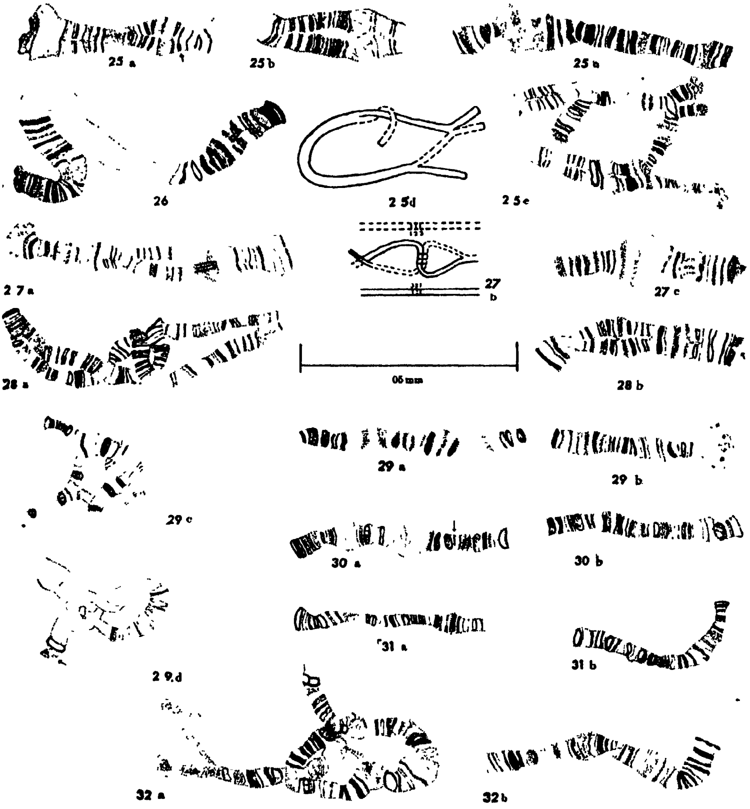
25 a
25 b
25 c
26
2 5d
2 5e
2 7a
27 b
27 c
28 a
05 mm
28 b
29 c
29 a
29 b
30 a
30 b
2 9.d
31 a
31 b
32 a
32 b

PLATE V

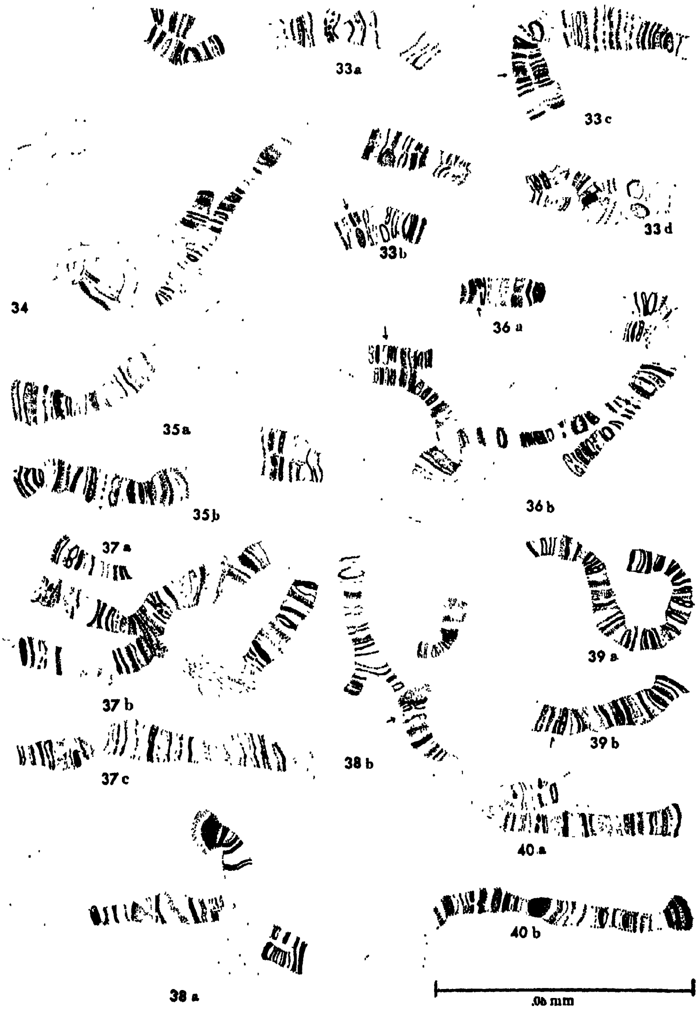
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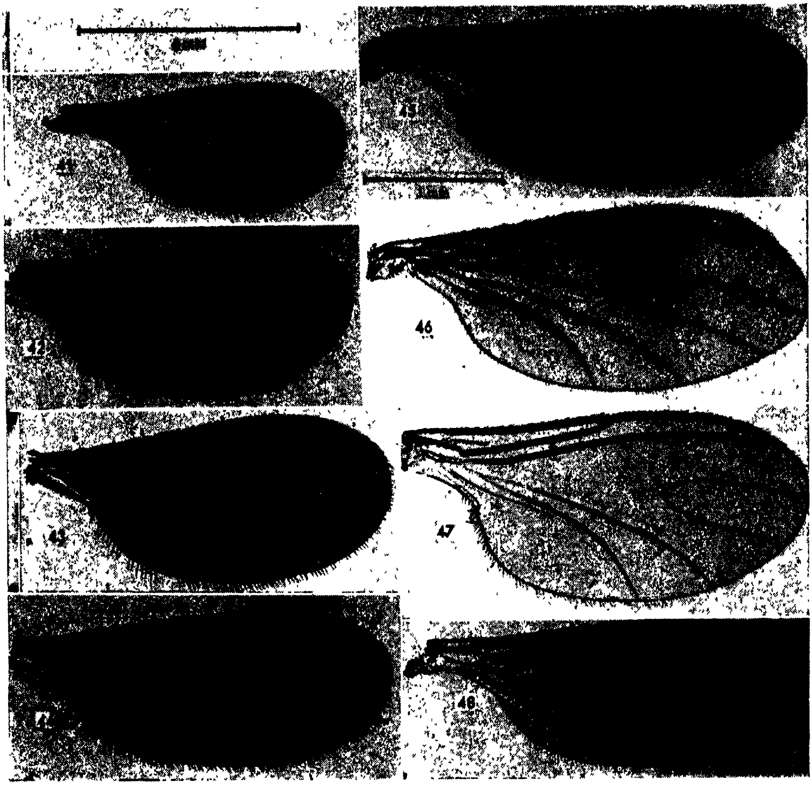
PLATE VI



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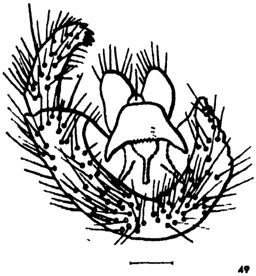
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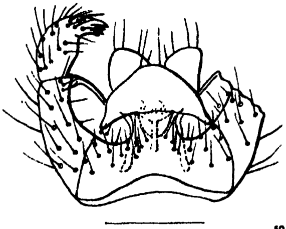
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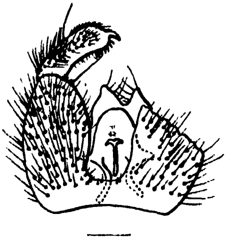
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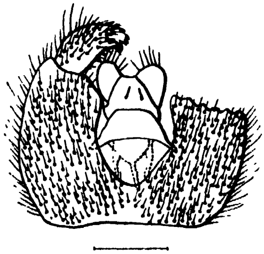
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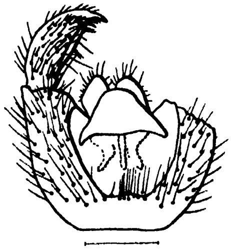
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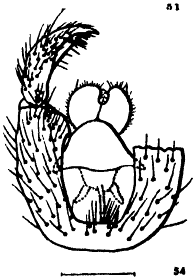
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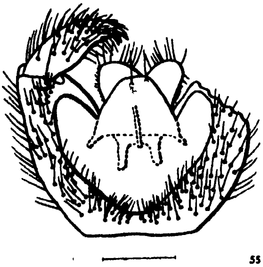
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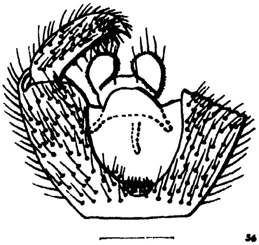
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PHENOGENETIC STUDIES OF THE HOMOEOTIC MUTANTS OF *DROSOPHILA MELANOGASTER*

III. THE EFFECTS OF TEMPERATURE ON THE EXPRESSION OF BITHORAX-34E

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THE mutant bithorax (chromosome 3, locus 58.8, symbol bx), the first homoeotic mutant of *Drosophila melanogaster* to be discovered, was described by Bridges and Morgan (1923). The phenotype of bithorax is very variable and overlaps the wild type. In flies which show the bithorax condition the metathoracic segment resembles the normal mesothoracic segment: an extra thorax and scutellum appear as an outpushing covered with hairs and bristles between the normal mesothoracic scutellum and the abdomen; the halteres are enlarged and either inflated and vesiculate or flattened and wing-like, with typical venation and large costal bristles. Bridges stated that in particularly favorable specimens the bristles which appear on this metathoracic outgrowth can be homologized exactly with the normal mesothoracic bristles, the dorso-centrals, post-alars, supra-alars, etc. Six other alleles of bx have since been found which vary in their dominance, viability and degree of expression. In this study the allele bx-34e, found by Schultz in 1934, was used because its expression is higher and more constant than the original bx.

A review of the phenomenon of homoeosis and its bearing on questions of comparative anatomy, embryology and evolution has been given elsewhere (Goldschmidt, 1940; Villee, 1942c). From the data on the development of the antennal discs in normal and aristapedia flies presented by Balkashina (1929), Goldschmidt (1938, 1940) derived a hypothesis of the action of homoeotic loci in producing their effects. He postulated that all the imaginal discs have identical potencies very early in development. Each becomes mature (or "competent" (Waddington)) at a certain stage in development different from the others. A series of evocators, one for each type of disc, is given off by the germ, each at the proper time to affect the proper disc which has just become mature and cause it to develop in the normal way. The homoeotic mutants upset this timetable so that a particular disc becomes affected by an evocator other than its normal one and so is differentiated into a structure abnormal for that location, *e.g.*, an antennal disc develops as a leg, or the labial bud develops as a leg or an arista. Balkashina found that the normal antennal disc begins to segment in the 4- or 4½-day larva, whereas the aristapedia antennal disc begins to segment in the 2-day larva, soon after the leg discs begin to segment. According to Goldschmidt, sometime during or before the second day of larval life an evocator is given off which diffuses into the six leg discs and causes a rhythmic subdivision of the distal end of the anlage, producing normal tarsus segmentation. Normally only leg discs are competent at this time and react to this induction, but any disc which happens to be competent will react by tarsus formation. The aristapedia gene, therefore, speeds up the maturation of the antennal disc, and causes it to become competent in the 2-day rather than the 4-day larva, so that the tarsus evocator present in the 2-day larva may induce it to form a tarsus.

Astauroff (1930) and Villee (1942b) reported the marked effect of extreme temperatures during development on the penetrance of tetraptera and tetraltera, respectively. In tetraptera, a homoeotic mutant resembling bithorax, there was a direct relationship between temperature and penetrance, the penetrance being about 1 per cent. at 17° C. and about 35 per cent. at 25° C. In tetraltera, in which the wings are changed to halteres, there was an inverse relationship between penetrance and temperature, the penetrance varying from less than 1 per cent. at 29° C. to over 35 per cent. at 14.4° C. Other studies on aristapedia and aristapedia-Bridges (Villee, 1942a, 1943a, b) and proboscipedia (Villee, 1943d, 1944) revealed that heat treatments (29° C. and 35° C.) given at various periods of development shifted development of the antenna of aristapedia flies toward the arista phenotype, while cold treatments (14.4° C.) shifted development toward the tarsus phenotype, whereas heat treatments shifted the development of the labial buds of proboscipedia flies toward the tarsus phenotype and cold treatments shifted development toward the arista phenotype. The present study was undertaken to determine the effects of heat and cold treatments on the development of the metathoracic discs of bithorax larvae. A preliminary report was given previously (Villee, 1943c).

MATERIALS AND METHODS

A study of the phenotypes of flies from the bx-34e stock and from preliminary heat and cold experiments showed that a graded series occurred ranging from a normal haltere (called type 1) to a large, flat, wing-shaped structure complete with four longitudinal veins (type 7). The series is practically identical with that described by Astauroff (1929) for tetraptera. Type 2 resembles a haltere, but the distal segment (head) is enlarged and bears a row of costal bristles. In type 3 the head is larger and beginning to flatten in the middle. Type 4 is larger and flatter than the preceding and has more bristles and hairs. Type 5 is large, flat and wing-like but has no wing veins. Type 6 is again larger and shows one or two longitudinal veins while type 7 has four longitudinal veins. The metathoracic wing is never more than about half the size of the normal mesothoracic wing.

Large numbers of male and female bx-34e flies were placed in half-pint culture bottles containing the usual cornmeal-agar-molasses medium and left for 2-hour egg-laying periods. The parents were then removed and the cultures kept at 22° C. until the temperature treatments were applied. After the treatments, the cultures were returned to 22° C. until the imagos hatched.

a. Effect of heat treatments on the development of bithorax-34e. To test the effect of high temperatures during development, groups of three cultures each were prepared as described above and placed in an incubator regulated at 29° C. beginning 0, 1, 2, 3, 4 or 5 days after the parents were removed and continuing 1 to 6 days. A total of thirty-three groups of three bottles each were prepared and given heat treatments of different durations begun at various times after the eggs were deposited. Following the treatment the cultures were returned to 22° C. Six cultures were kept at 22° C. as controls and another group of eight cultures were kept at 29° C. throughout development. The adult flies which

TABLE 1

EFFECT OF 29° C. TREATMENTS ON THE EXPRESSION OF BITHORAX-34E

Days of development Treatment		Bithorax types						Total	Code
Begun	Ended	1	2	3	4	5	6		
0	1	..	7	13	..	..	..	20	265
0	2	..	17	89	25	..	..	131	306
0	3	..	1	45	3	..	..	49	304
0	4	1	6	80	23	..	..	110	314
0	5	..	6	24	10	..	..	40	310
0	6	..	2	31	7	..	..	40	312
1	2	1	53	141	2	..	..	197	272
1	3	1	43	209	3	..	..	256	284
1	4	..	4	103	49	1	..	157	330
1	5	1	9	133	50	..	..	193	320
1	6	..	5	77	13	..	..	95	309
1	7	1	9	132	19	..	..	161	305
2	3	..	42	92	6	..	..	140	274
2	4	1	8	119	40	..	..	168	317
2	5	..	6	60	24	..	..	90	320
2	6	..	7	95	31	1	..	134	320
2	7	..	1	31	18	..	..	50	334
2	8	..	..	116	38	..	..	154	325
3	4	..	38	110	..	..	..	148	274
3	5	..	33	150	17	..	..	200	291
3	6	..	..	3	1	..	..	4	325
3	7	..	..	7	2	..	..	9	322
3	8	..	..	9	3	..	..	12	330
3	9	..	..	6	4	..	..	10	340
4	5	..	10	8	2	..	..	20	260
4	6	..	6	62	44	..	..	112	334
4	7	..	1	76	36	..	1	114	334
4	8	..	2	43	5	..	..	50	306
4	9	..	..	9	3	..	..	12	325
4	10	..	..	2	8	..	..	10	380
5	6	..	1	6	..	..	..	7	286
5	8	..	..	5	..	..	..	5	300
5	9	..	..	6	6	..	..	12	350
						Grand total		2,910	

hatched from the treated larvae were classified as to type of metathoracic appendage and counted. The results of the counts of the three bottles in each group were combined and are presented in Table 1. To simplify comparisons between treatments, the system of code numbers devised earlier (Villee, 1943b) was used. The percentage of flies of each type present in each of the experimental groups was calculated. The code number is the sum of the products of the percentage of flies of each type times the respective type number (1 to 7). The code number, therefore, lies between 100 (if 100 per cent. of the group were type 1) and 700 (if 100 per cent. of the group were type 7). By this means a single number represents the effect of a particular treatment on the development of the metathoracic appendage in bithorax. The mean code number of the control group kept at 22° C. throughout development and its standard deviation was 267 ± 6.5. The mean code number and standard deviation of the group kept at 29° C. throughout development was 302 ± 10.5.

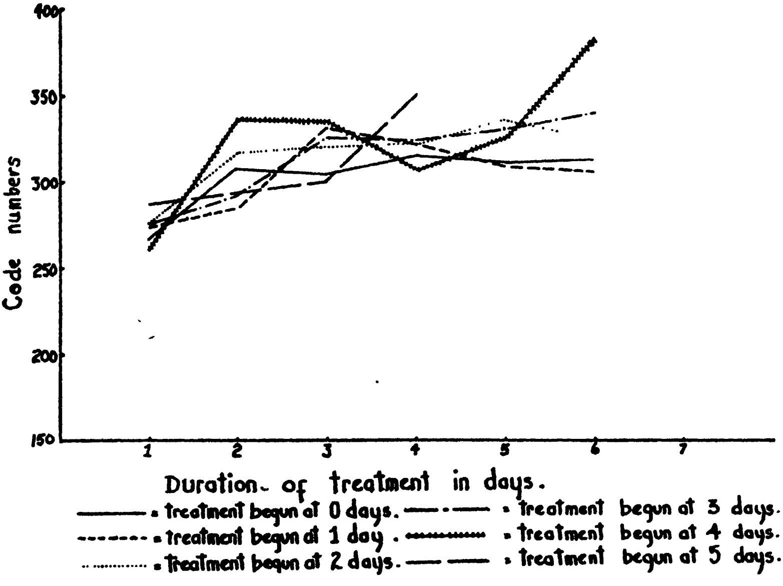

FIG. 1. 'Variation in the effect of heat treatments on the expression of bithorax-34e (given in code numbers) with the duration of the treatments. Each curve represents treatments begun on a particular day of development.

In Fig. 1 these code numbers have been plotted against the duration of the treatments in days to present graphically a comparison of the effectiveness of the various treatments in increasing the expression of the mutant

TABLE 2

EFFECT OF 15° C. TREATMENTS ON THE EXPRESSION OF BITHORAX-34E

Days of development Treatment		Bithorax types				Total	Code
Begun	Ended	1	2	3	4		
0	1	..	..	16	..	16	300
0	2	..	11	88	..	99	289
0	3	..	27	101	..	128	279
0	4	..	77	126	..	203	262
0	5	1	74	60	..	135	243
0	6	..	92	34	..	126	227
0	7	..	54	29	..	83	235
1	2	..	13	74	..	87	285
1	3	..	8	67	3	78	294
1	4	..	86	58	..	144	240
1	5	..	64	78	..	142	255
1	6	..	77	31	..	108	229
1	7	..	33	15	..	48	231
2	3	..	12	40	2	54	282
2	4	..	3	33	..	36	292
2	5	..	43	66	..	109	261
2	6	..	22	13	..	35	237
2	7	2	67	37	..	106	233
2	8	24	29	2	..	55	159
3	4	..	13	88	1	102	288
3	5	..	43	97	2	142	272
3	6	..	17	31	1	49	267
3	7	..	60	61	4	125	255
3	8	..	15	19	..	34	256
3	9	1	4	..	..	5	180
3	10	4	45	1	..	50	194
3	11	2	16	..	..	18	189
4	5	..	14	9	..	23	239
4	6	..	6	5	..	11	245
4	7	1	49	41	1	92	243
4	8	..	41	15	..	56	227
4	9	1	17	3	..	21	209
4	10	5	26	..	..	31	184
4	11	1	7	1	..	9	178
4	12	2	21	..	..	23	191
5	6	..	..	18	3	21	314
5	7	..	42	56	..	98	257
5	8	2	81	41	..	124	232
5	9	..	106	30	..	136	222
5	11	1	39	13	..	53	223
5	13	2	57	6	..	65	206
						Grand total 3,080	

(the greater the transformation of the haltere into a wing, the higher the code number). The increase in expression is roughly proportional to the length of the treatment up to a maximum of three or four days, after which a continuation of the treatment has little or no effect in increasing the expression. Treatments begun four days after egg deposition were slightly more effective in increasing expression, *i.e.*, in causing a shift in development toward a wing rather than a haltere, than those begun earlier or later. The code number of the control group, 267 ± 6.5, may be used in judging the effectiveness of the several treatments.

b. Effect of cold treatments on the development of bithorax-34e. Cultures of bx-34e eggs were prepared as

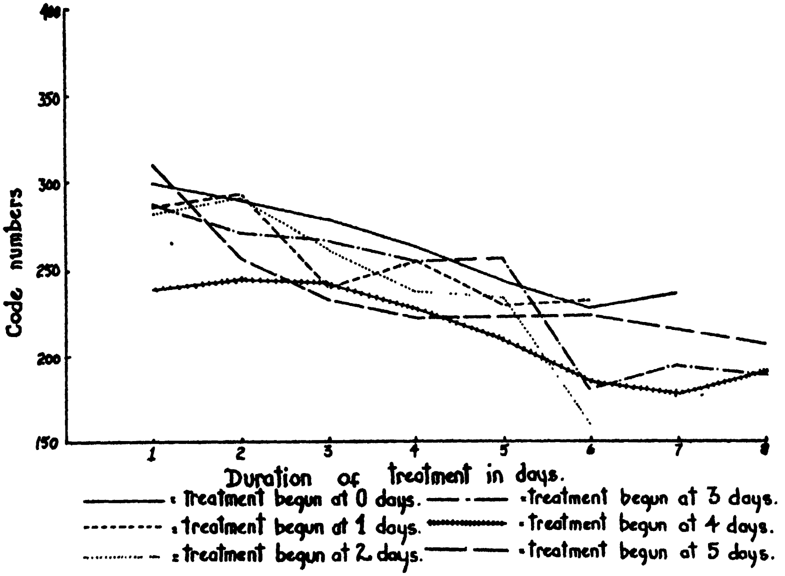

FIG. 2. Variation of the effect of cold treatments on the expression of bithorax-34e (given in code numbers) with the duration of the treatments. Each curve represents treatments begun on a particular day of development.

described above and placed in groups of three in an incubator regulated at 15° C. beginning 0 to 5 days after egg deposition and continuing 1 to 8 days. A total of forty-one groups of three bottles each were prepared and subjected to low temperatures during various periods of development. After the treatments the cultures were returned to 22° C. to complete development. The adult flies which hatched were classified as to metathoracic appendage and counted. The results of the counts of the three cultures comprising each group were combined and are given in Table 2 together with the code numbers derived from the figures. These code numbers were then plotted against the duration of the treatments in days (Fig. 2) to show graphically the relative effects of the different treatments in decreasing the expression of bithorax (the less the haltere has been transformed into a wing, the lower the code number). The decrease in expression again is roughly proportional to the duration of the treatment. Treatments begun four days after the eggs were laid were most effective in decreasing the expression, those begun three or five days were less effective and those begun at other times were still less effective in decreasing the expression of bithorax, *i.e.*, in causing a shift in development toward a haltere instead of a wing. The code number of the control group, 267 ± 6.5, may again be used as a base line in judging the effectiveness of the several treatments.

Discussion

The finding that high temperatures increase the expression of bx-34e, causing an increase in the shift in development toward the wing phenotype, and low temperatures decrease this, causing development to shift toward the haltere phenotype agrees with the results of Astauroff's (1930) experiments with tetraptera and Villee's (1942b) work with tetraltera. In all three, high temperatures shift development of the meso- or metathoracic disc toward the wing phenotype and low temperatures shift development toward the haltere phenotype. Since the embryological processes resulting in the bithorax or tetraptera phenotype, the changing of a haltere anlage to produce a wing, are presumably opposite to those resulting in the tetraltera phenotype, the changing of the wing anlage to produce a haltere, the opposite relationship between temperature and penetrance or expression in the stocks is indicative. The action of temperature on development in general is to accelerate or retard differentially certain processes in relation to others, since the chemical reactions involved will probably have different temperature coefficients. Villee (1942b, c) assumed, therefore, that the production of a wing instead of a haltere requires the differential acceleration of some process or processes which is increased by higher temperatures during or before the determination and differentiation of the wing and haltere discs; whereas the production of a haltere instead of a wing requires the differential retardation of these same or similar processes which is increased by lower temperature. These data do not enable us to tell what part or parts of the developmental system the temperature treatments are affecting. They may be affecting the rate of maturation of the meso- or metathoracic discs, the rate of production of the evocator substance, or something else. The study of proboscipedia (Villee, 1944) showed that the effects of temperature treatments were best explained by supposing that they affect the rate of maturation of the imaginal discs and probably the same holds for bithorax.

Chen (1929) and Auerbach (1936) found that the wing bud develops from the central part of the dorsal mesothoracic disc during the latter part of the fourth day of larval development and that the first five prepupal hours are critical for wing formation. The wing everts during the sixth prepupal hour. The haltere bud is recognizable at the end of the larval period (4–4½ days) and is completely formed and segments during the first five prepupal hours. Robertson (1936) found that the haltere bud evaginates a little later than the wing bud. We may therefore assume that the mesothoracic evocator is released before the metathoracic evocator. If the reverse should later be found to be true, the following hypothesis would not be invalidated but could still be true after minor changes in the details.

We may now apply the theory suggested by Goldschmidt (1938, 1940) outlined previously and the modifications suggested by Villee (1944) to explain the action of tetraltera, tetraptera and bithorax and the effects of temperature on their development. If we assume that in the normal, wild-type fly, each disc matures at the proper time to be affected by the normal evocator for that disc, we may call this rate of maturation 1. The dorsal mesothoracic disc normally becomes competent when the mesothoracic evocator is released and responds to the induction by the formation of a wing. The metathoracic disc normally becomes competent a little later, when the metathoracic evocator is released, and responds by the formation of a haltere (Fig. 3). If we now assume that the bithorax and tetraptera genes alter the rate of maturation of the metathoracic disc so that it is greater than 1, the metathoracic disc will become competent faster, hence earlier, and will be affected by the mesothoracic evocator and respond by the formation of a wing and a bristle-covered structure resembling a scutellum and mesonotum. Similarly the tetraltera gene may alter the rate of maturation of the mesothoracic disc so that it is

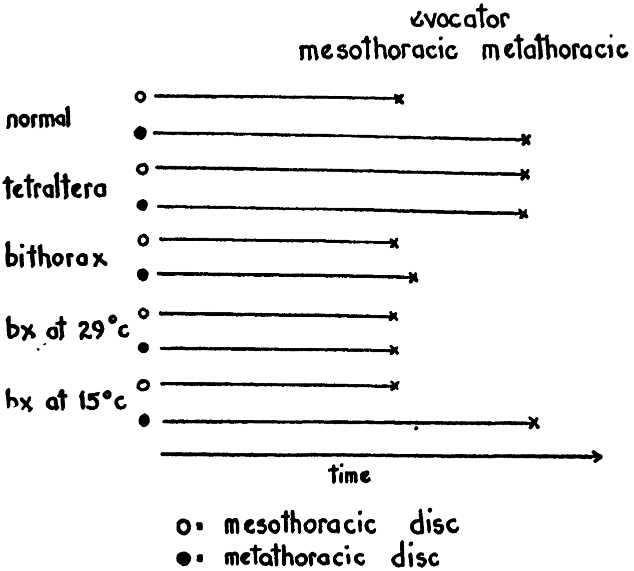

FIG. 3. Scheme of the interactions of maturation and evocator release to produce normal or abnormal induction of the dorsal meso- and metathoracic discs. See text for discussion.

less than 1 (less than its normal rate, whatever that may be), so that the mesothoracic disc becomes competent later than normal, is affected by the metathoracic evocator and responds by the formation of a haltere and a metathoracic-like dorsal mesothorax.

The temperature treatments affect this system by accelerating or decelerating the rate of maturation of the discs differentially from the rate of development as a whole. In bithorax, we assume that the mutant gene changes the rate of maturation of the metathoracic disc, speeding it so that it becomes competent earlier in development than normal. High temperatures applied during development will speed all chemical reactions but will speed those leading to the maturation of the metathoracic disc more than the others because the bx gene has altered the rate of development of the metathoracic disc relative to the rate of development as a whole. This additional effect of temperature will thus cause the metathoracic disc to become competent even earlier and be affected more strongly by the mesothoracic evocator, and causing a greater shift towards the wing phenotype than at normal temperatures. Conversely, low temperatures applied during development will slow down all chemical reactions but will slow those leading to the maturation of the metathoracic discs more than the others. The temperature treatments will thus cause the metathoracic disc to become competent relatively later in development; in extreme cases, so late that it is not affected by the mesothoracic evocator but by the metathoracic one. Similar considerations explain the effect of temperature on the penetrance of tetraptera and tetraltera.

It is unnecessary for this hypothesis that the evocators be diffusible substances produced in one part of the body (Goldschmidt's "germ") and diffusing into the imaginal discs, resembling the eye color hormones which are produced in the fat bodies and Malpighian tubules and diffuse into the eye discs. The evocators may be substances of a molecular size too large to diffuse through cell membranes which are produced in the nucleus of each cell of an imaginal disc (as well as in all other cells of the body) by some particular locus, and released to the cytoplasm when the nuclear membrane breaks at mitosis.

The fact that bithorax affects the whole metathoracic disc as well as the haltere bud has been noted. The metanotum is changed into a structure resembling a mesonotum and scutellum on which are bristles similar to those on the normal structures. The temperature treatments affected the development of these structures as well: low temperatures during development caused them to be very small or absent, high temperatures during development caused them to be large and covered with many bristles. An extension of the explanation offered above would cover this phenomenon as well.

The phenomenon investigated here is an example of the "branching-track" sort of development discussed by Waddington (1940). The branching is not so definite as in aristapedia or proboscipedia, where the intermediate forms have one part definitely arista-like and another tarsus-like with a sharp transition line between. In bithorax the intermediates have some wing and some haltere characteristics, with a complete series of intergrades between wing and haltere. These intermediates may indicate that the metathoracic evocator is released shortly after the mesothoracic evocator so that intermediates are easily produced, whereas tarsus and arista evocators are given off at quite different times in development so that induction occurs one way or the other, with a sharp line of transition between. In terms of Waddington's "epigenetic landscape," the valleys are not so deep nor the cliffs so high in the branching between wing and haltere as between tarsus and arista so that development may fail to go in one valley or the other but may go somewhere between. Other possible explanations involve thresholds which differ progressively along the length of the disc in aristapedia but which are absent in bithorax, or some essential difference in the ability of the imaginal discs to react.

SUMMARY

Bithorax-34e flies were given high and low temperature treatments during development, beginning 0 to 5 days after the eggs were laid and continuing 1 to 8 days. Heat treatments increased the expression of the mutant, causing an increase in the change of the haltere to a wing. Cold treatments decreased the expression, causing the metathoracic appendage to be more haltere-like. The increase or decrease was roughly proportional to the duration of the treatment and treatments begun four days after egg deposition were slightly more effective in shifting development than those begun earlier or later. The treatments affected the development of the extra thorax and scutellum which appear between the normal scutellum and abdomen in a similar fashion. The application of Goldschmidt's theory of the action of homoeotic loci to these data is discussed.

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PROLONGATION OF LARVAL-PUPAL DEVELOPMENT IN *DROSOPHILA MELANOGASTER* AND ITS EFFECT ON FACET NUMBER[1]

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INTRODUCTION

THERE are several instances in the literature in which the effect of prolongation of developmental time and the resultant effect on gene expression have been reported. Only a few of these reports were concerned with facet number in *Drosophila melanogaster.* Braun (1939) and Green and Oliver (1943) investigated the effect of prolongation of development in the larval stage on the expression of genes for wing characters in *Drosophila melanogaster.* They postulated the elaboration of a substance by the factors involved. Prolongation of development enhanced the expression of the responsible factors by allowing additional time for elaboration of the substances which resorb the already fully formed material that would normally form the character as in the case of notched wing as studied by Braun. Green and Oliver, in brief, report on experiments with vestigial wing in which they found that prolongation of developmental processes resulted in an increased expression of the vestigial character due to an increase in the amount of substance elaborated by the factor responsible. Luce (1939, 1940, 1942) studied the effects of formalin, of oxygen and of a mixture of oxygen and carbon dioxide on facet number in bar alleles of *Drosophila melanogaster.* He found (1942) that high concentrations of formalin increased the length of development and reduced the mean facet number. In this instance the lengthening of development enhanced the expression of the bar factors. The oxygen studies of the same author conducted on a bar infrabar strain (1940) produced different results. The time of development was prolonged, but the expression of the gene difference at the bar locus was decreased as the facet number of the flies was greater than in the controls. His studies on the effect of a mixture of 95 per cent. oxygen and 5 per cent. carbon dioxide (1939) yielded similar results in an infrabar strain.

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[1] A part of a thesis entitled "The Physiology of the Development of the Imaginal Buds in the Bar Series of *Drosophila melanogaster*" submitted for the degree of doctor of philosophy in zoology in the Graduate School of the University of Illinois, 1944.

VITAMIN B_1 (THIAMIN HYDROCHLORIDE)

The present study was carried out upon bar and ultrabar strains of *Drosophila melanogaster* and dietary deficiencies were utilized to induce prolongation of development. Schader (1941) noted a prolongation of larval development due to a diet deficient in vitamin B_1. Keenan, Kline, Elvehjem and Hart (1935) devised a technique for inactivating vitamin B_1 in fresh cake yeast. Van 't Hoog (1935) formulated a synthetic culture medium for insects which could be prepared and kept sterile. Baumberger (1919) found that microorganisms were a source of nutrition to insects. He also showed that autoclaved yeast was a completely adequate food for *Drosophila melanogaster.*

VITAMIN E

There is no conclusive evidence that insects need any other vitamin than vitamin B for normal development. Hill and Burdett (1932) believed, however, that the royal jelly of honey bees, which is the substance necessary for the transformation of worker larva to the queen or sexually mature form, was a rich source of vitamin E. They interpreted their results as indicating the presence of appreciable amounts of the vitamin in royal jelly, but subsequent investigations by Mason and Melampy (1936), Evans, Emerson and Ackert (1937), and Haydak and Palmer (1939) failed, after careful assay, to reveal any vitamin E in this substance. The utilization of vitamin E inactivation methods (Waddell and Steenbock, 1928) were employed on the culture medium used in part of this study to determine if this vitamin is essential in *Drosophila* growth and development.

MATERIAL AND METHODS

The strains of flies, bar and ultrabar, used in this experiment had a sufficiently reduced number of facets so that the "error factor" in counting facets was greatly minimized. These strains were originally derived from a bar-eyed line inbred since 1923, at a constant temperature of 27° C. This is the so-called "temperature-inheritance line" of the University of Illinois.

The culture medium used for the stock flies from which individuals were obtained for the diverse experiments is a modification of the Russian formula by Luce (unpublished). It comprises these ingredients:

80	ml	biological water (chlorine free)
1.5	gm	agar agar
1.5	"	dried brewer's yeast
6.0	"	yellow corn meal
6.0	"	syrup
5.0	"	ground raisins
		a small amount of powdered yeast foam to each vial

The water, agar agar and corn meal were heated and allowed to boil until the ingredients were almost cooked. The brewer's yeast, syrup and raisins were then added, and all were permitted to boil for several minutes. The food was then placed in 25 × 100 mm shell vials, about one inch of food to each vial. Powdered yeast foam was sprinkled lightly on the food surface after it had cooled and solidified.

VITAMIN E DEFICIENCY

The vitamin E inactivated medium was obtained by treating the corn meal and raisins of the medium with ferric chloride and ether (Waddell and Steenbock, 1935). One per cent. by weight of ferric chloride, which had been powdered, mixed with a quantity of ether equal to the volume of the food treated was the mixture used for inactivation of the vitamin E contained in the food. The oxidizing medium and the food treated were kept in a closed vessel for 24 hours to permit extraction of the fat soluble vitamin and the oxidation of it. After 24 hours, the ether was allowed to evaporate by removing the cover of the vessel. After complete evaporation of the ether, the treated food was ready for use in the preparation of the experimental medium. To check on the possibility of lethal effects or toxicity due to the presence of the ferric chloride, a second medium was prepared which contained an amount of ferric chloride equal to that used in the inactivation process.

Vitamin B_1 Deficiency

A vitamin B_1 deficient medium was prepared synthetically (Van't Hoog, 1935). It contained agar agar, sucrose, yeast and Pearl's salt mixture, modified by Sang. The salt mixture consisted of three different solutions:

Solution A

8.00 gm	potassium sodium tartrate
2.00 “	ammonium sulfate
0.50 “	magnesium sulfate
250.00 ml	distilled water

Solution B

5.00 gm	tartaric acid
0.65 “	potassium dihydrogen phosphate
250.00 ml	distilled water

Solution C

0.25 gm	calcium chloride
250.00 ml	distilled water

The solutions were autoclaved for 20 minutes at 250° F. and under 15 pounds of steam pressure. In the preparation of the experimental medium, 10 grams of Fleischmann's fresh cake yeast was added to a flask containing 25 ml of distilled water. This was autoclaved for 5 hours at 250° F. and under 15 pounds of steam pressure. This treatment inactivated the thiamin hydrochloride present in the yeast (Keenan, Kline, Elvehjem and Hart, 1935).

Schader (1941) and others have found that this inactivation process also affects the potency of riboflavin and nicotinic acid present in the yeast. Because of this it was necessary to add these vitamin B components in the proportion of 14.3 mg of nicotinic acid and 0.05 mg of riboflavin to each 100 ml of the culture medium. The autoclaved yeast was added to 25 ml of solution A and 25 ml of solution C. The nicotinic acid, riboflavin, 1.5 grams of agar agar and 1 gram of sucrose were added to this mixture. Twenty-five ml of solution B were contained in another flask. The two flasks were autoclaved for 20 minutes at 250° F. and under 15 pounds of steam pressure. Solution B was then mixed with the contents of the other flask and the resultant mixture was boiled for one minute. Three ml of this mixture were transferred to sterile cotton-stoppered, 25 × 100 mm shell vials by means of sterile pipettes. The food was allowed to cool and solidify. It was important that the steps as outlined be followed, since hydrolization of the agar agar occurred if this precaution was not observed. In preparing the control and check media, the same procedure was observed except that in the control the yeast was not autoclaved for more than twenty minutes. Also the riboflavin and nicotinic acid were not added. In a check medium experiment the culture medium consisted of the experimental medium plus an amount of thiamin considered to be equivalent to that inactivated. The thiamin added was contained in a sterile aqueous solution. The amount added was 0.08 mg/100 ml of culture medium.

PARENT FLIES AND EGG COLLECTING

The stocks of flies used in the study had been closely inbred at a constant temperature of 24° C. ± 0.5°. From these stocks pure male and female ultrabar and bar flies were selected. Eight males and 8 females were placed in each vial containing the stock culture medium. They were inbred for several more generations and then used for this study.

The method of collecting eggs is original and permits of securing large numbers in a short time. It also facilitates handling of eggs in transferring them to vials. Strips of absorbent paper-toweling were cut to fit into the shell vials. Onto these paper strips a 1 per cent. agar agar solution was dropped in the shape of oval buttons. After the agar had solidified, the "buttons" were grooved with a needle, painted with a solution of 2 parts of glacial acetic acid, 10 parts sorghum molasses and 88 parts distilled water. The paper strips were then transferred to the shell vials containing 8 pairs of the stock flies to be used for parents. The vials containing the strips of paper were placed in a lidded, metal chamber lined with moistened absorbent paper. Thus all the factors conducive to greater egg production were supplied. The ovipositing period was of four hours' duration and the age of the egg-larva could be determined with a – 2 hour error if the midpoint of the egg-laying period was used as the time of oviposition (Alpatov, 1929).

To collect the eggs the agar "buttons" were placed in a Petri dish after removal from the paper strips. They were observed under a binocular microscope and a determination of the number of eggs on each "button" was made. The eggs were rendered aseptic as follows. A sufficient volume of 85 per cent. alcohol was poured into the Petri dish to cover the "buttons." This treatment destroys any yeast cells which might be clinging to the eggs but does not injure the embryo within (Schader, 1941). According to Baumberger (1919), yeast cells are not transmitted from the adult female to the eggs. This procedure greatly obviated the possibility of contaminating the culture media used in these experiments. After one hour in 85 per cent. alcohol, the agar "buttons" were transferred to vials with a sterile dissecting needle.

TEMPERATURE CONTROL, ASEPSIS AND CALCULATIONS

The experiments of the present study were all carried out at the optimum temperature for *Drosophila melano-*

gaster development, *i.e.*, 24° C. ± 0.05°. This was accomplished by the use of a constant-temperature chamber specially constructed and well insulated containing both cooling and heating units under automatic control. A thermograph made constant recordings of the temperature.

Aseptic conditions were maintained throughout the study by autoclaving all cultural media, food vials, pipettes, etc. The vials used for flies were flamed whenever transfers were made, the eggs were placed in 85 per cent. alcohol for one hour, and all contaminated vials were immediately discarded.

Hourly observations were made during the critical developmental periods and a record of time spent in larval and pupal development was obtained. The facets of the compound eyes of random samples of flies obtained from these experiments were counted under an E. Leitz, Ultrapak microscope. The random sample consisted of 20 to 40 individuals of each sex for each culture medium used. Statistical constants were obtained from these samples.

EXPERIMENTAL RESULTS

The experimental results are presented in tabular form comparing length of developmental times, the mean of the facet number for males and females, standard deviation of the mean and standard error of the mean. Statistical significance is also indicated in these tables.

In all the experiments conducted the rate of mortality, the fertility and the sex ratio of the flies were essentially normal.

DISCUSSION

The data obtained from this study are significant in several respects. From a nutritional viewpoint it is evident that thiamin hydrochloride is not necessary to complete development though it is necessary for normal development. This would be in accord with the results of Lafon's findings (1935) and Schader's (1941). A study of Table 1 would indicate that the amount of thiamin added to the culture medium which had been subjected to vitamin B_1 inactivation was not equivalent to the amount inactivated, for we find an extension of the developmental periods where this medium is employed as well as a significant increase in facet number. It can be concluded that this medium was partly deficient in thiamin, which resulted in a reduced effect on both lengthening of development and facet number as compared to complete inactivation of thiamin in the other experimental medium.

TABLE 1

Prolongation of Larval and Pupal Stages of Development Due to Vitamin B_1 Deficiency and a Consequent Increase in Facet Number in a Bar Strain

		I Control	II Experimental organisms cultured on B_1 inactivated food to which .08 mg of B_1/100 ml of culture medium was added	III Experimental organisms cultured on a medium treated for inactivation of Vitamin B_1
Mean larval period in hours		190	202	323
Mean pupal period in hours		110	120.5	167
Facet number	Males	132.10	150.45	162.28
	Females	83.10	87.31	95.00
Standard deviation of the mean	Males	± 5.27	± 3.67	± 4.75
	Females	± 6.32	± 2.92	± 4.55
Standard error of the mean	Males	± .96	± .62	± .91
	Females	± 1.38	± .61	± .91
Statistical significance	Males	+ with II	+ with III	+ with I
	Females	+ with II	+ with III	+ with I

(+ = statistical significance.)

It is not presumed that the inactivation of vitamin E was the causative factor in prolonging development in the ultrabar strain studied. The report of Tatum (1939) and some unpublished work by the author indicates that no other vitamin than vitamin B is essential to *Drosophila* development. It is evident that the addition of ferric chloride to the culture medium had no significant toxic effect. It is concluded that the vitamin E inactivation process had an effect on the lipids present in the food treated. It is altogether probable that some fat-fraction, yet unidentified, was oxidized by the treatment. Since the chief concern of this study is the factors which influence gene expression, it does not matter which food component is actually affected by the inactivation process.

TABLE 2

PROLONGATION OF LARVAL AND PUPAL STAGES OF DEVELOPMENT DUE TO VITAMIN E INACTIVATION AND A CONSEQUENT INCREASE IN FACET NUMBER IN AN ULTRABAR STRAIN

		I Control	II Experimental organisms cultured on a medium containing 1 per cent. ferric chloride by weight	III Experimental organisms cultured on a medium treated for Vitamin E inactivation
Mean larval period in hours		167	178	272.50
Mean pupal period in hours		120	131	154.00
Facet number	Males	29.27	29.88	31.97
	Females	26.00	27.19	29.27
Standard deviation of the mean	Males	± 4.20	± 2.58	± 2.76
	Females	± 2.42	± 2.42	± 3.36
Standard error of the mean	Males	± .66	± .40	± .46
	Females	+ .46	± .46	± .67
Statistical significance	Males	– with II	+ with III	+ with I
	Females	– with II	+ with III	+ with I

(+ = statistical significance, - = absence of significance.)

The absence of thiamin and the inactivation process of vitamin E had no appreciable effect on the mortality rate, fertility or sex ratios, but they did slow up the physiological and metabolic activities which led to normal development. This slowing up with a consequent prolongation of development had a peculiar effect on the expression of the factors responsible for facet number. The expression of these gene-differences at the bar locus was decreased rather than enhanced. Braun (1939) and Green and Oliver (1943) postulated an enhancement of gene expression for the characters studied when the developmental time of the larval stage was prolonged. This enhancement of expression was reflected in a more pronounced effect on the end-characters than when development was normal.

The conclusions of Braun are based on the hypothesis that the notched-wing character he studied was due to a resorption by substances elaborated by the gene for the character. The longer the time of development, the longer the time the gene has to act and hence the greater the amount of resorptive substance elaborated. Green and Oliver suggest that from the data they have obtained it would seem that the gene for vestigial wing belongs in the category of antimorphic genes. This would mean that the gene for vestigial acts to prevent normal differentiation of wings. Prolongation of development permits an increase in the time of gene action so that in the case of heterozygous *vg* sufficient substance to cause scalloping is produced. The quantity of vestigial substance elaborated depends upon the dosage of *vg,* the allele present and the extent of prolongation of the third larval instar.

It is evident from the results of the present study that the gene differences at the bar locus do not express themselves in a manner comparable to the factors studied by the above-mentioned authors. In the case of eye development, a study of the embryology has shown that there is no evidence that resorption takes place at any time (Steinberg, 1941). Also, from the data obtained it is not possible to associate the mode of gene expression for facet number with that for vestigial. In the latter case the prolongation of development enhanced gene expression, while in the former case it was decreased. If the gene differences for facet number at the bar locus were enhanced in their expression, then a greater reduction in facet number would result, but in the present study we have found that the facet number was increased by the prolonged developmental time.

It can be postulated that the factors for facet number are slowed in their expression by prolongation of the larval period which results in an increased number of facets. This postulation is bolstered by the investigations of Luce (1939, 1940 and 1942) as well as by the results of numerous investigations on temperature effects on *Drosophila melanogaster.*

SUMMARY

A diet deficient in thiamin hydrochloride greatly prolongs the larva-pupa time of development with a consequent effect on facet number in a bar strain of *Drosophila melanogaster.*

A culture medium treated for inactivation of vitamin E by use of a mixture of ferric chloride and ether not only has the vitamin E inactivated but some other food component which is thought to be a fat-fraction. This treated medium considerably prolongs larval-pupal development with a consequent effect on facet number.

The facet number of flies cultured on both types of culture media was increased significantly. This would indicate a decrease of gene expression due to the prolongation of development caused by the dietary deficiencies.

From these data it can be concluded that the factor responsible for facet number in *Drosophila melanogaster* does not function in a manner comparable to the so-called antimorphic gene *vg* which was studied by Green and Oliver (1943) nor the gene for notched-wing which elaborates resorptive substances as postulated by Braun (1939).

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REVIEWS AND COMMENTS

EDITED BY PROFESSOR CARL L. HUBBS

IN these reviews and notices of current biological publications emphasis is given to books and major articles which fall within the special scope of THE AMERICAN NATURALIST, in that they deal with the factors of organic evolution. REVIEWS AND COMMENTS are meant to include also such general discussions, reports, news items and announcements as may be of wide interest to students of evolution. Except as indicated, all items are prepared by Dr. Carl L. Hubbs, Scripps Institution of Oceanography, University of California, La Jolla, California. All opinions are those of the reviewer.

Tempo and Mode in Evolution. By GEORGE GAYLORD SIMPSON. New York: Columbia University Press, 1944: i–xviii, 1–237, figs. 1–36. $3.50.

THIS newest first-rank treatise on evolution may be characterized as brilliant, but not flashy; brimful of fresh ideas for the researcher, but hardly fitting the needs of an elementary student; comprehensive, though making no pretense at covering the whole field of organic evolution. It is neither a textbook nor a compendium—several of each have recently appeared—but rather the vehicle by which the gifted author presents the philosophical conclusions, regarding the rates and methods of evolution, which he has reached during the course of his extensive researches and deliberations in paleontology. Though the background and base data are largely those of a paleontologist, a broad and objective viewpoint has been sought and rather consistently attained. Usually with success and wisdom the fossil record is continuously viewed in the light of the facts and interpretations of modern genetics, as well as from the eyes of a paleontologist. As a result Simpson, unlike many of his colleagues, quickly sets aside all Lamarckian principles, dispenses with metaphysical concepts of inner drives and strivings for perfection, and merely annotates as inacceptable to him such common ideas as those of "exuberant racial youth" and of "racial old age." He treats as overgeneralizations the dicta of some paleozoologists that all evolution is adaptive, that all evolution has definite trend and that evolution is never reversible, although it is clear that he regards these general principles as having usual validity.

As one would expect of a coauthor of the sound and thorough treatise, Quantitative Zoology, Simpson repeatedly quantifies data on the tempo and mode of evolution —judiciously and with due reservations. Some approximations thus reached are very interesting and suggestive. For example, it is estimated that gene mutations may be expected to occur simultaneously in an individual only once during a time period four times the age of the earth! (Perhaps Goldschmidt will take this as evidence either of the evolutionary inadequacy of gene mutation or of the non-existence of genes.)

Though he avers that "the materials for evolution and the factors inducing it are believed to be the same at all levels," and therefore joins many other biologists in attacking Goldschmidt's sharp distinction of microevolution (within species) and macroevolution (between "good" species), Simpson in passing uses not only "micro-evolution" and "macro-evolution" but further proposes a new etymological hybrid, "mega-evolution," to refer to the large-scale phylogeny of major groups. He explains that most paleontologists regard such major evolution as more distinct from macroevolution as treated by Goldschmidt, than macroevolution is from microevolution. In a way he outdoes Goldschmidt, for he has not only expanded on that geneticist's classification but has also presented a three-fold break-down of evolution on each of two additional bases. In the second classification, with an apology for the introduction of the new terms, he distinguishes rates and hence kinds of evolution (and the phyletic lines involved) as *bradytelic* (low-rate, as in "arrested evolution"), *horotelic* ("standard-rate," whatever that may be) and *tachytelic* (high rate). As a third classification, referring to modes, evolution is typed into (1) *speciation,* characteristic of low taxonomic levels; (2) *phyletic evolution,* typical of groups from genera to families but not strictly confined to these ranks;

and (3) *quantum evolution,* principally exemplified in the higher taxonomic categories, and therefore roughly corresponding with "mega-evolution," though the modified Goldschmidtian classification seems to have been forgotten in the final arrangement. Many biologists will see no fundamental qualitative difference between bradytelic and horotelic rates or lines and will fail to appreciate the need for such terms. "Speciation" has some distinctive features and will surely remain in use, largely because it is the aspect of evolution that is subject to genetic research and that is at present the evolutionary field of chief concern to most taxonomists. "Phyletic evolution," emphasizing consistent trends, and "quantum evolution," focused on the diversified early development of a group or on the rapid transition from one "adaptive peak" to another, are useful points of view, but seem to me to apply almost equally to all taxonomic levels and to intergrade completely. I would regard them as stages in phylogeny, "quantum" preceding "phyletic," rather than as different sorts of evolution.

Lest it appear that I disagree widely with Simpson I have compiled and present below with enthusiastic approval a series of his statements, selected to represent his evolutionary philosophy (sentences and phrases have been rearranged, with some deletions and with some conjunctions added):

> The extent and nature of variability are themselves important group characters subject to natural selection and other evolutionary factors. Segregation or selection of intragroup [intraspecific] variability can give rise to new groups at a potentially rapid evolutionary rate. Evolution on the basis of existing variability cannot proceed beyond about the specific level. In continuous phyletic evolution of large populations at moderate rates variability tends toward a relatively constant level. Progressive loss of variability does not appear to be a usual or general principle of evolution; loss of hereditary variation is balanced by the appearance of new mutations. Rate of sustained evolution normally shows little or no correlation with variability, but conservative groups are sometimes exceptionally variable. Extreme variability is more likely to appear in degenerating than in rapidly progressing structures.
>
> Mutations with extremely small phenotypic effects that occur at moderate or low rates, such as have repeatedly been found in experimentation, are consistent with [observed] rates of evolution; their tempo and mode are not

> necessarily or primarily controlled by mutation rates. Paleontological evidence does not require saltation.
>
> The appearance of a mutant individual is not evolution. Only populations, not individuals, evolve. However profound or systemic a mutation may be, it is difficult to see how its eventual effect on populations can be independent of the neo-Darwinian factors. The sudden appearance of a really new individual reaction system morphologically of generic or higher grade would not produce a new genus or higher genetic group unless and until this system came to characterize an isolated population.
>
> Many phyletic lines progress in a regular manner toward a condition that has appeared to some students so fixed as to be predestined. "Orthogenetic" evolution, however, is almost invariably adaptive. Observed rectilinear sequences are usually most consistent with the theory that orthoselection is the dominant or primary factor, and some are not logically explicable in any other way. There are several ways in which momentous effects can arise within the scope of the neo-Darwinian theories of mutation–selection. There is no good evidence that a trend has ever been continued by momentum beyond a point of advantageous or selectively neutral modification or has ever been the direct cause of extinction. The most general explanation is simply that a phylum has become highly specialized and adaptively specialized and a change in selective influences has made the specialization secondarily inadaptive: lag, but not momentum.
>
> Selection is a truly creative force. It is one of the crucial determinants of evolution. New groups invariably represent a major ecological adaptive change. Characters hitherto supposed to be nonadaptive are quite definitely adaptive and characters of unknown adaptive value are nevertheless definitely selected by differential survival. In large populations evolution is extremely slow except under the influence of selection, and then it is almost purely adaptive in type and approximately proportional to selection intensity in rate.

On the combined basis of paleontological and relative-growth data "four basic theorems concerning rates of evolution" are thus stated:

> 1. The rate of evolution of one character may be a function of another character and not genetically separable even though the rates are not equal.
> 2. The rate of evolution of any character or combination of characters may change markedly at any time in phyletic evolution, even though the direction of evolution remains the same.
> 3. The rates of evolution of two or more characters within a phylum may change independently.
> 4. Two phyla of common ancestry may become differentiated by differences in rates of evolution of different characters, without any marked qualitative differences or differences in direction of evolution.

Some other principles of phylogeny are stated by Simpson in the following words:

> The usual sequence is for one dominant group to die out, leaving the zone empty, before another group becomes abundant. For most animals the ex-

panding phase is shorter than the contracting phase so that groups narrowly distributed are more likely to be relicts than new or expanding groups, but relatively restricted area characterizes both the earliest and the latest stages of phyletic history. The best part of the paleontological record is made up of lines that evolve in approximately one direction over long periods of time. It is, however, obvious that rectilinear evolution is far from universal. More specialized branch phyla tend to evolve more rapidly than their parent stocks and more specialized phyla tend to become extinct before less specialized: the rule of the survival of the relatively unspecialized. Phyla evolving more slowly or not evolving except for small and apparently random variations sometimes continue indefinitely, even for hundreds of millions of years, in this evolutionary state of rest.

In view of the fact that the book was completed and put through the press while the author was in military service it seems gratuitous to point out what appear to be minor defects. It is to be hoped, however, that the next edition will carry a summary of the author's views, and will give greater attention to such pertinent zoological data as explosive evolution in unsaturated Recent habitats. The chapter on Organism and Environment—the least satisfying to the reviewer—might be revised. Some details of terminology and of organization might be reconsidered, though as a whole, the text is lucid and remarkably free of errors. What fellow workers will most want in the next edition or in a supplementary treatise are more facts and interpretations from the author's great store and some consideration of evolutionary problems that are given little or no emphasis in the present volume.

The Dice of Destiny. An Introduction to Human Heredity and Racial Variations. By DAVID C. RIFE. Columbus, Ohio: Long's College Book Co., 1945: 1–163, figs. 1–23. $1.75.

AFTER being surfeited with rebuttals of the Nazi philosophy of racial superiority, all based either on the almost equally unscientific idea that human races do not differ at all in mental characters or on the still more absurd notion that races do not exist, we find real relief in the appearance of a scientifically sound treatise on human race problems. The human biologist shows himself able to avoid the pseudo-science that seems to pervade in part the thought of many physical anthropologists as well as sociologists.

After introducing his little book as a treatment on politico-genetics, as contrasted with medical genetics, Rife proceeds to demonstrate, in words that may be understood by those untrained in genetics or any other phase of biology, how individuals differ genetically in "a matter of taste" (to phenyl-thio-carbamide), in blood antigens, in hair, eye and skin color, in various other physical features, in dermatoglyphics, in handedness, in mental capacity, in special abilities; further, how racial groups are characterized by different frequencies of the genes responsible for the physical and mental make-up of the individual. It is shown that other characters are not consistently correlated with Nordic blondness, that Jews are superior in some respects, that no race has a monopoly of desirable characters, that racial differences are quantitative not qualitative.

Interspersed with this line of presentation are simplified discussions of human heredity, devoid of any cytogenetic detail, and of sex ratios and of traits associated with sex. There is also a biologically sound treatment of the interplay between heredity and environment, each of vital and indispensable importance in determining the characteristics of individuals and hence of racial and national groups.

Toward the end of the book the conclusions of human biology are increasingly linked with ethics to build up a democratic philosophy of individual and racial conduct. Though in such discussions the author may have transgressed the limits of science he has not overstepped our current ideals of human relationships.

Dice of Destiny is a book of great potential value, an antidote to the poisons of Nazi philosophy and to the unfounded rebuttals that have flooded the public. It should pass through several editions, which, incidentally, would provide means for correcting such minor defects as typographical errors and occasional passages that are not clear.

The Distribution of the Birds of California. By JOSEPH GRINNELL AND ALDEN H. MILLER. Pacific Coast Avifauna (Cooper Ornithological Club, Berkeley, Calif.), 27, 1944: 1–608, frontisp., figs. 1–57. $6.00 (unbound), $7.00 (bound).

INFINITE precision has been the obvious watchword in the preparation of this large volume on the distribution of the birds of California. For each of the 644 kinds of birds verified as of natural occurrence within the state limits there is given, for California, the reproductive and migratory status, with an indication of abundance; the geographic range in elaborate detail, supported by a great wealth of references; and the habitat. For the commoner species there are stated the habitat features that appear to be of significance in determining occurrence and distribution. Systematic discussions are limited to notes explaining the status that is accepted by the authors. Blind adherence to the American Ornithologists' Union Check-list is avoided and condemned. I note also a criticism of the "freely exercised vogue for joining in one species related geographically complementary forms."

Of particular value are the 57 excellent maps showing, for the breeding season, the record stations and distribution of each subspecies within California, for the species (with a few exceptions) having two or more named races in the state. It is recognized that the maps as prepared fail to delimit areas of intergradation and conceal the discontinuities within ranges. Failure to identify and to spot map intergrades I regard as a major defect not only of this treatise but also of ornithological work in general, and makes one wonder how far "authority" still rules in the science of birds.

The treatment is rigidly confined to the state of California. Small insert maps showing the entire distributional limits of the forms breeding in California would have greatly increased the value and utility of the book.

This source book, of infinite detail, will be indispensable to all students of the birds of western North America, and will be valuable also to workers in ecology, zoogeography, game management and speciation. Conspicuously absent, however, is any synthesis of the data: no maps of life zones, communities or faunal districts of the state; no principles regarding zoogeographical patterns, differentiation centers, barriers, size of ranges, types of intergradation, etc.; no generalizations on the ecological and habitudinal control of distribution. Naturalists in many fields will eagerly await the fulfillment of the promise that such syntheses will be presented in a separate work.

NOTICE OF NEW BOOK

Amphibians and Reptiles of the Chicago Area. By CLIFFORD H. POPE. Chicago: Chicago Natural History Museum, 1944: 1–275, pls. 1–12 (6 in color), figs. 1–50. $1.75.—Like many another youth Clifford Pope idolized the late Raymond Ditmars, read and reread his works on reptiles, but unlike the rest, Pope grew up to replace his former mentor as America's foremost author on popular herpetology. He has the uncommon ability to present scientific material in an informative, fascinating, and unstilted style without sacrifice of authenticity.

It is gratifying to find that emphasis is placed on a general life-history account of each species, including habits, reproduction, growth, age, food, enemies, importance, habitat, occurrence, and maintenance in captivity. Keys and recognition characters are adequate, but identification is treated as a tool, not an end. Each of the 52 species is well illustrated, 16 in color, and references are appended.

Although prepared primarily for residents of the Chicago area, this work will fill a general need, especially in the North Central states region. Readers of general interests will find in it a great fund of well-organized, reliable information. Every herpetologist will want it on his desk.—REEVE M. BAILEY, *Museum of Zoology, University of Michigan, Ann Arbor, Mich.*

SHORTER ARTICLES AND DISCUSSION

A MORE MEANINGFUL FORM OF THE "LOGISTIC" EQUATION

EQUATIONS of growth are legion; even those which are called "logistic" occur in a great variety of forms (see, for instance, Kavanagh and Richards, 1934; and Wilson and Puffer, 1933). Of these, perhaps the most attractive to the rational biologist is:

$$N = \frac{K}{1 + e^{a-bt}} \tag{1}$$

which results from the integration of:

$$\frac{dN}{dt} = bN\frac{K-N}{K} \tag{2}$$

where:

N = number of organisms (or any similar measure of growth or multiplication)

K = upper asymptote of N

b = "biotic potential" or potential rate of increase in the absence of environmental resistance

t = time

e = base of logarithms

The above differential equation, first applied (in somewhat different form) to the problem of biological growth by Robertson, 1908, and by Ostwald, 1908, owes its attractiveness to the fact that it includes only terms which refer to identifiable entities, combined in accord with assumptions which seem reasonable to the experimental biologist. The rationale behind the equations has been clearly expounded by Gause, 1934, whose symbols I use. It will be noted, however, that during the course of integration the expression picks up the arbitrary constant a. To those who hold with Jacques Loeb (see Osterhout, 1928) that "all the observed phenomena [of biology] should be expressed in the form of equations containing no arbitrary constants," this constant, a, constitutes a blemish in the logistic function. From such a point of view it is desirable to eliminate the offending member. This can be done as follows:

Consider the value of the function when $t = 0$, i.e., when $N = N_0$.

$$N_0 = \frac{K}{1 + e^{a}} \tag{3}$$

$$e^{a} = \frac{K}{N_0} - 1 = \frac{K - N_0}{N_0} \tag{4}$$

$$a = \ln \frac{K - N_0}{N_0} \tag{5}$$

Having evaluated *a* we may write:

$$N = \frac{K}{1 + e^{\ln\left(\frac{K - N_0}{N_0}\right) - bt}} \tag{6}$$

which, by the definition of logarithms, is:

$$N = \frac{K}{1 + \left(\frac{K - N_0}{N_0}\right) e^{-bt}} \tag{7}$$

Thus the arbitrary constant is replaced by an expression involving only the asymptote of the growth function (K) and the initial value (N_0). That *a* can be evaluated was recognized by Gause in his treatment of graphical methods (p. 149); but he nevertheless retained the less meaningful constant in his logistic equation. Undoubtedly others who have actually worked with logistic equations have also realized the significance of *a,* but little or no overt notice appears to have been taken of the situation.

Whether this substitution will be convenient or not will depend on the nature of the problem attacked. In the case of natural populations, the value of N_0 needed for a "good fit" may sometimes be obviously false, due to the gross accidents of fortune that attend the establishment of new enterprises. In that case one might prefer to retain the more cryptic symbol *a* in the exponent; or, one might use another common form of the equation:

$$N = \frac{K}{1 + Ce^{-bt}} \tag{8}$$

where: $\ln C = a$.

With experimental material, however, which permits the direct determination of N_0, equation (7) should be preferred. In fact, the possibility of employing this more meaningful form of the growth equation may be regarded as a test of the validity of the logistic "law" and of the degree of the investigator's control of the experimental situation.

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INDUCTION OF OVOTESTIS IN ESTROGEN TREATED ADULT MALE TELEOST, XIPHOPHORUS HELLERI

IN a study on sex-differentiation in the teleost, *Xiphophorus helleri*, Essenberg (1923, 1926) found that 50 per cent. of the young females transformed to males before sexual maturity was reached and two cases of natural complete sex-reversal were observed in parturient females. He concluded that any agent or condition which tends to decrease the capacity for female sex hormone secretion beyond a certain limit becomes an immediate factor in sex-reversal in the female of this species. This postulation was substantiated by the administration of testosterone propionate, which caused not only the morphological masculinization but accompanied by developing of a male gonad with spermatogenesis in approximately 50 per cent. of the experimentally treated *Xiphophorus* females (Baldwin and Goldin, 1939, 1941). The appearance of changes in secondary sex characters of the female *Xiphophorus,* although no clear testicular differentiation was noted in the ovary, by treatments of androgens has also been reported (Witschi and Crown, 1937; Regnier, 1938). Moreover, Witschi (1942) mentioned the unpublished results of his student Crown (1941) that many *Xiphophorus* females became masculinized by treatment of estrone; other cases of complete sex-reversal were observed in these females when treated with gonadotrophin from pregnancy urine (Baldwin and Li, 1942).

While the sexuality of female *Xiphophorus helleri* appears to be unstable and changeable, detailed investigation on the actions of estrogenic hormones on the male fish of this species is highly important before one can come to a solution regarding the sexual development of the fish. Regnier (1938) reported that injections of estrogen into *Xiphophorus helleri* and *Lebistes reticulatus* suppressed the male secondary sex characters and inhibited the spermatogenesis, but the formation of ovocytes in the testes has not yet been reported. However, Berkowitz (1938, 1941) has shown that estrogen feeding induced a mosaic of male-female characters in the male *Lebistes reticulatus* and produced a marked inhibition of the testis together with its transformation into an ovotestis, and almost complete reversal of testis to ovary was observed, whereas injection of estrogens caused less reversal and egg cells rarely appeared. We have continued to study the influence of estrogen in mature male *Xiphophorus* to determine if possible to what extent this form can respond in a comparable way to the results reported to occur in other species.

Experimental. Two series of adult male fish, besides the control and other experimental groups, were injected retroperitoneally with alpha-estradiol benzoate (Progynon-B) :[1] one series of 25 fish each received a weekly dosage of 0.03 mg; and the other series of 37 fish each received the same amount of estrogen at intervals of 15 days. The experiment was conducted over a period of 18 months, and the treated animals were sacrificed for histological observations after varying lengths of administrations. In general, there were no estrogenic effects on the already well-developed gonopodium and sword of the caudal fin, although perhaps there was a slight fading in general bodily coloration. The testes showed different degrees in hastening of spermatogenesis, atypical release of spermatozoa, hypertrophy of interstitial tissue, atrophy of sperm ducts, tubules and the gonad as a whole, and other pathological conditions. A detailed study of these responses will be reported elsewhere. The present communication notes two cases of ovotestis which were induced in the second series of treated animals that after the treatment had been discontinued for some time.

The first ovotestis was observed in a fish (MPB 41) which had received 12 injections and was killed 4 months after the last injection. The hypertrophied sperm ducts were distended with a large number of spermatophores and formed the main bulk of the exhausted gonad. The thin cortical portion consisted of sperm tubules, which appeared destitute of germ cells, and scattered cysts of gametogonial cells; neither spermatocytes nor spermatids were noted. The epithelium of these ducts and tubules were more or less atrophied, and there were increasing amounts of interstitial tissue. Three small ova were found in the middle part of the elongated gonad. One oocyte, measured 80 μ by 65 μ in diameter, was located between the fused edges of the two gonadal lobes (Fig. 1). The other two, measured 40 μ by 52 μ and 65 μ by 78 μ in diameter respectively, were clustered beneath the external epithelium of the gonad. The vesicular nuclei of these oocytes contained clearly defined nucleoli, but no yolk granules were formed in the cytoplasm. Some cysts of large gonial cells were always associated with these egg cells.

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[1] The authors wish to thank Dr. Max Gilbert, of the Schering Corporation, for the Progynon-B.

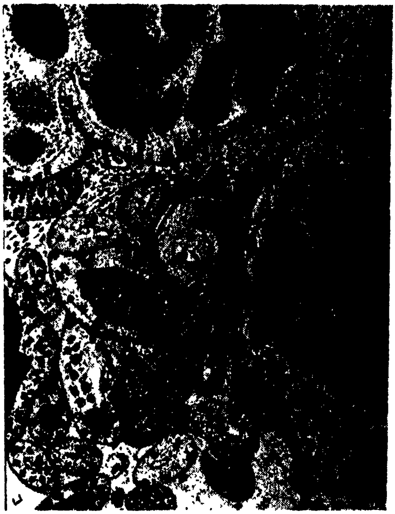

FIG. 1. Section of part of an ovotestis (MPB 41) showing an oocyte among the atrophied sperm ducts and tubules. × 220.

Another ovotestis was found in a fish (MPB 71) in which treatment had been discontinued for 6 months after it had received 24 regular injections. Histological examination (Fig. 2) revealed that the gonad resembled an immature testis with large cysts of various stages of spermatogenic cells except the mature sperm. The small centrally placed gonoducts were devoid of any germ cells and no spermatophores were found. The sperm tubules were extremely rare. At least 12 small ova were counted in the central part of this gonad and many of them showed a certain degree of disintegration in their nuclear structures. The diameters of these egg cells were reduced, ranging from 105 μ by 78 μ to 65 μ by 40 μ or even smaller. The follicular epithelium was also disorganized, hence, most of these ova were merged indistinctly into the surrounding spermatogenic cells. Other cell structures were similar to those as described in the previous case. The scarcity of interstitial tissue was very conspicuous in this gonad. It seemed that the rejuvenized testicular tissue would eventually overrun the degenerating ova.

Discussion. Our results show that there is a capacity for the development of ovotestis in the mature male *Xiphophorus helleri* when the animals had undergone prolonged treatments of small doses of estrogens. Continuous administration of high doses of estrogens, however, were not effective in developing egg cells, because they caused a marked suppression in the development of germ cells and the atrophy of testicle. These effects are somewhat similar to those found in the estrogen treated male *Lebistes reticulatus* (Berkowitz, 1941). The gametogonia which lie upon the periphery of the ovotestis appear to be the immediate source in the formation of ova. It is interesting to note that there is no instance of reversal of the testis in adult male amphibians either naturally or by experimental procedures as the "male cortex" disappears in older males so that they lose their amphisexual character of the larval gonad (Humphrey, 1942).

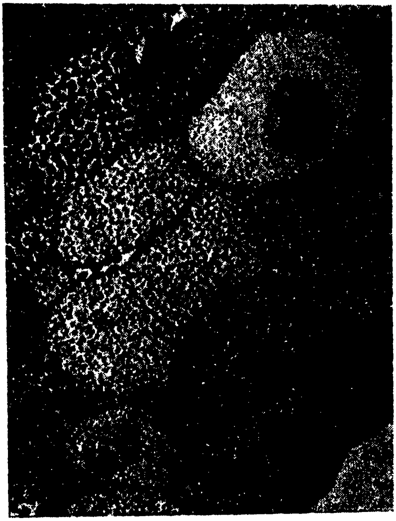

FIG. 2. Section of part of a second ovotestis (MPB 71) showing oocytes and cysts of spermatogenic cells. × 308.

Essenberg (1926) claims that in *Xiphophorus helleri* sex is determined and controlled by the sex hormones derived from the ovary and testes; chromosomes are not considered as having the function of sex determination and control. Ralston (1934) notes 24 tetrads in the first maturation division of this species and suggests that one of the large chromosomes which takes its position on the equatorial plate slower than the others, and advances quickly its precocious divided daughter components to the poles of the spindle, represents a sex chromosome. He interprets the condition as one in which the male is homogametic and infers the sex chromosomes of *Xiphophorus* as the ZZ–ZW type. In a similar cytological study, however, Friedman and Gordon (1934) have not confirmed the differentiation of sex chromosomes in this species. Unfortunately, sex-linked factors have not been discovered in *Xiphophorus helleri.* Thus far the genetic investigations upon this species have not yielded conclusive results concerning the type of sex-determining mechanism. From the cross-breeding experiments between *Xiphophorus helleri* and *Platypoecilus maculatus,* which is known as ZZ–ZW type, Kosswig (1935) concludes that the sex of *Xiphophorus* is determined phenotypically by environmental influences. Goldschmidt (1937) reinterprets Kosswig's results, assuming somewhat a ZZ–ZW type ($FFMM_1 = ♀$; $FFMM = ♂$) in line with the work done upon the undifferentiated races of frogs by Witschi. Whatever the hereditary mechansim for sex determination may be, or even without any such mechanism as indicated by Witschi (1939) and others, the tendency for maleness is dominating in *Xiphophorus helleri;* this is supported not only by the facts in their sexual development but also by the experimental administrations of sex hormones. There is no complete sex reversal from male into female observed in the adult male of this species and the ovotestis which is induced by estrogen treatments may gradually change back to a testis when the hormone is withdrawn.

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THE EFFECTS OF LOW INCUBATION TEMPERATURES ON THE EXPRESSION OF ROSE AND DUPLEX COMB, CREST, MUFFS, FRIZZLED, NAKED NECK AND TRIPLE SPURS IN THE DOMESTIC FOWL

STURKIE (1943) demonstrated that polydactyly in the fowl could be suppressed by lowering the temperature of incubation at certain stages of development. Most of the treatments were employed upon embryos from 2 to 5 days of age, and, in most cases, before differentiation of the character. Warren (1944) also suppressed polydactyly with low temperatures, but not to the extent as that reported by Sturkie. The treatments which were effective in suppressing polydactyly, and others, were used in an attempt to modify the expression of rose and duplex comb, crest, muffs, frizzled, naked neck and triple spurs. All these characters are unifactorial-dominant ones.

The stages of development at which the low temperatures were applied varied, but an attempt was made to employ the treatments at what was considered the probable critical period for the characters; however, this was not the case for a number of treatments, particularly those employed at two, three and five days on crest, duplex combs and muffs, since these treatments were designed and used mainly for the effect on polydactyly.

A Houdan male, homozygous for crest, duplex comb and muffs, was mated to normal White Leghorn females. Likewise a homozygous frizzled male was mated to non-frizzled females. Thus all the embryos or progenies of these matings under control conditions were expected to exhibit, and did show the dominant characters, except in the case of the frizzled mating where a few normal or questionable offspring were obtained. The rose-comb, triple-spur and naked-neck males which were mated to normal females were heterozygotes and hence a 1:1 ratio was expected and obtained in their progeny under control conditions.

TABLE 1

THE EFFECTS OF LOW TEMPERATURE UPON THE EXPRESSION OF ROSE AND DUPLEX COMB, CREST, MUFFS, FRIZZLED, NAKED-NECK AND TRIPLE SPURS

Age of embryo treated Days	Treatment	Character and no. classified	
Two	16 –48–R[1] 3.3°–34–C	Crest, Duplex comb, Muffs	94*
	124 – R 34°– C	Rose Comb 6 : Naked Neck Single Comb 12 : Normal	13 8
Three	12 –96–R 10°–34–C	Rose Comb 5 : Naked Neck Single Comb 5 : Normal	14 17
	16 –48–R 34°–34–C	Crest, Duplex Comb, Muffs	79
	96 – R 34°– C	Triple Spur Normal	7 3
Five	12 –96–R 3.3°–34–C	Crest, Duplex Comb, Muffs	11
	96 – R 34°– C	Triple Spur Normal	10 7
Six	96 – R 34°– C	Triple Spur Normal	6 6
	12 –60–R 3.3°–34–C	Crest, Duplex Comb, Muffs	17
Seven	12 –72–R 3.3°–34–C	Crest, Duplex Comb, Muffs	14
Eight	12 –72–R 3.3°–34–C	Crest, Duplex Comb, Muffs	9
Nine	16 – R 3.3°– C	Rose Comb 20 : Naked Neck Single 20 : Normal	23 18
Ten	16 –72–R 3.3°–34–C	Frizzled Non-Frizzled	74 4

* Progeny showing contrasting characters were not obtained.

[1] 16 hours at 3.3° C. then 48 hours at 34° C. and the remainder of incubation period at control temperature (37.5° C.).

The results of the treatments upon the expression of these characters are shown in Table 1. It is obvious that these treatments did not affect the expression of any of the characters except possibly the treatment on rose comb at two days. In view of the results of treatments at three and nine days on this character, it is not believed that the treatment at two days accounts for the shortage of rose-combed progeny thus obtained. The percentage of non-frizzled progeny obtained after treatment was no greater than that secured under control conditions, and these may have resulted from the action of a modifying gene or genes for frizzling such as reported by Landauer (1933) and Hutt (1936), or from an error in classification.

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GENES AS PHYSIOLOGICAL AGENTS[1]

GENERAL CONSIDERATIONS

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MY function in this symposium is to sketch in a background for the concrete topics to be presented by the others.

It is appropriate to consider first what a gene is, especially as it has been questioned recently whether the concept has not outlived its usefulness (Goldschmidt, 1940). A gene is primarily, of course, merely a hypothetical entity postulated to make sense out of the results of breeding experiments. The time has long passed, however, since there has been need for any hesitation in identifying the mendelian units with regions of concrete chromosomes. A mendelian gene is a block of self-duplicating chromosomal material that is not divided as far as observed by the process of crossing over or by the chromosome breakage, associated with rearrangement.

There is no necessary implication that the gene is an absolute entity or that it is the smallest unit capable of self-duplication. A study of the effects of multiple alleles suggests some degree of physiological unity as the rule, but there are interesting exceptions which suggest that the essential pattern may often be replicated along the chromosome and that such "repeats" within the gene tend to drift apart in complementary fashion, by a well-known evolutionary mechanism, to become at length distinct genes, separable by crossing over and chromosome breakage (*cf.* Lewis, 1941; Oliver and Green, 1944). Estimates of average gene size, on the hypotheses that all the material in a Drosophila sperm head is genic, give figures of the order of 10^{-16} grams (Morgan, 1922; Muller, 1929) corresponding to a molecular weight of 6×10^7 and thus some thousand fold that of a typical small protein, though comparable to the so-called giant molecules of certain proteins (hemocyanin) and the crystallizable nucleo-proteins of viruses (Stanley, 1943). Most investigators find the condensed chromosomes of sperm cells to consist almost wholly of basic proteins (protamine or histone) combined with highly polymerized desoxyribose nucleic acid (*cf.* Mirsky and Pollister, 1943).

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[1] Symposium of the American Society of Zoologists, Cleveland, September, 1944.

It has been maintained that both of these are too simple in chemical composition to provide an adequate basis for the practically infinite diversity of genes. The possibilities of stereoisomerism are, however, almost unlimited. Moreover, there is now evidence of a direct sort that desoxyribose nucleic acid actually can transmit from one organism to another the potentiality for indefinitely continued synthesis of itself and also of a chemically unrelated specific substance. Avery, McLeod and McCarty have recently obtained an extract from one specific type of pneumococcus, apparently consisting of pure polymerized desoxyribose nucleic acid (molecular weight about 500,000), which can transform unencapsulated cells from another type into the type from which the extract was obtained. The desoxyribose nucleic acid not only conveys the capacity to produce the specific polysaccharide on which type specificity depends but also the capacity to produce more of itself. As to the protein constituent, the spacing of amino acids in a polypeptide chain is practically the same as that of nucleotides in nucleic acid (3.34Å, Astbury, 1941) and there is the possibility of corresponding specificities based on stereoisomerism. It should be added that Stedman and Stedman (1943) have recently reported the presence of large amounts of a more complex protein of the globulin type in fish sperm.

The essential pattern of the gene can hardly be more than 2 dimensional to permit synthesis of a duplicate, except that, as noted, patterns that are in some sense complementary may be represented in a nucleic acid and a protein layer. It has been maintained that the essential pattern of the gene can be represented only once or at most twice in the direction of cleavage of the chromosome to account for the usual lack of mosaicism of mutant individuals, derived directly from x-rayed sperm in Drosophila (Muller, 1928, 1929; Timofeeff-Ressovsky, Zimmer and Delbrück, 1935). But there may be any number of parallel lamellae provided that these interact in such a way that a mutational change in one is either restored rapidly to the type of the others, or spreads through them all, a process which might conceivably occur from the same mechanism as involved in the duplication process. Demerec (1928) has described an exceptional case in *Drosophila virilis* in which an influence of this sort seems to extend to a particular mutable gene (reddish-a) from any stable allele (type, yellow or stable reddish) in the homologous chromosome. Reddish-a is stable except in such heterozygotes. (It may be noted that stable reddish and yellow may both be like type in the part of the pattern affected in reddish-a.) A gene may perhaps be comparable to a little crystal with many repetitions of molecular pattern which, however, tend to become differentiated except in the direction of cleavage.

In this diagram (Fig. 1), I have attempted to indicate the relation of genes to characteristics at successive levels of organization. Most people seem to be born preformationists. They tend to take for granted a separate heredity for each part of the adult organism. The treatment of homology in comparative anatomy often seems to have this implication. Even physiologists sometimes attribute a character partly to physiological factors, partly to heredity, as if heredity could operate by some sort of sympathetic magic, independently of physiological channels. The attitude of physiological genetics is that characters are determined 100 per cent. by physiological processes, but that genes are the ultimate internal physiological agents.

Genes can be thought to act directly only on the metabolic processes in the protoplasm immediately surrounding their positions in the cells which contain them. They can affect morphological characters only by determining one or another reaction of cells to local conditions. The relation of genes to organic structure does not differ essentially from that to hereditary extraorganic structure as of webs of spiders, nests of termites, wasps, birds, etc. As indicated in the diagram, a gene is related to a character through a chain of processes in which behavior at each level determines structure at the next, while this in turn gives a secure basis for behavior at the higher level (Wright, 1934a).

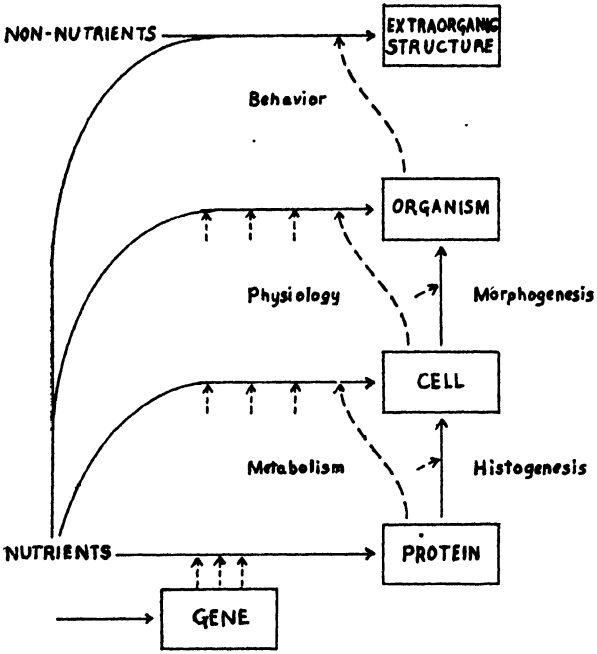

FIG. 1.

The most characteristic physiological action of a gene is the synthesis of another gene like itself. Let us consider what this involves under the prevailing conceptions of biochemistry. The reversible transformation of glucose into lactic acid ($C_6H_{12}O_6 \rightleftharpoons 2C_3H_6O_3$) has been shown to require about a dozen steps, each of which involves a separate enzyme system. To suppose that a highly specific giant nucleo-protein molecule is formed from nutrients by such a step-by-step process seems intolerably complex, even making due allowance for repetitions and for the possibility of catalytic combinations of successively larger blocks. I think that most geneticists have long agreed that there must be a short cut. The gene must somehow act as a model on which a daughter gene is formed as a whole. (Alexander and Bridges, 1928; Koltzoff, 1928; Haldane, 1935, etc.). This is not a mere desire for simplicity. The high degree of autonomy of genes and their capacity to duplicate as of the new sort after mutation are hardly compatible with any step-by-step process. Autonomy, is, to be sure, a relative matter. The enzyme trypsin acts as an autocatalyst in transforming the substance trypsinogen into more trypsin. But trypsinogen is probably as complex a molecule as trypsin. At the other extreme is the autonomy of a sulfur bacterium or a green plant which produces its own kind of material almost from the elements. We ourselves at a higher level of organization are intermediate in this scale and so apparently at a much lower level are genes and viruses. If the synthesis of a given gene depended on the prior synthesis of specific substances of increasing complexity, catalyzed by products of a succession of other genes, to which the parent gene contributes merely a final touch, we would expect to find that mutations at any given locus would bring about mutational changes at many other loci. A few cases are indeed known in which replacement of one gene by an allele tends to bring about mutation of another gene (Demerec, 1941; Rhoades, 1941), or of many others (Demerec, 1937; Mampell, 1943).

These cases show that genes are not always completely autonomous. Genes and viruses are indeed all probably dependent with respect to energy requirements. The rule, however, is a very high degree of autonomy with respect to constitution. It is difficult to avoid the conclusion that the pattern of forces emanating from a gene somehow tends in the living cell to arrange simple molecules on its surface in such a way as to duplicate its molecular pattern. The possibility of a reciprocal relation between steric patterns of polynucleotide and polypeptide chains has been noted. This process is represented diagrammatically in the upper portion of Fig. 2 for comparison with similar models for other aspects of immediate gene physiology.

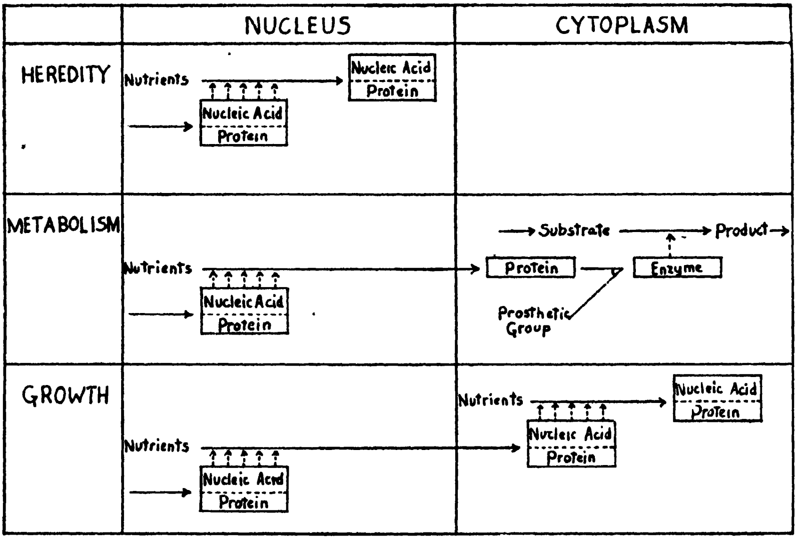

Fig. 2.

There is, of course, no doubt that numerous chemical transformations of small molecules occur in cells in the conventional step-by-step fashion. It is quite possible that the genes themselves may function as catalysts for intranuclear reactions. But in general such processes are carried out by enzymes which are certainly not the genes themselves. This is obviously true of the digestive enzymes which act outside cells and also of those which have been located in mitochondria and microsomes in the cytoplasm (Lazarow, 1943).

The enzymes as far as known are either pure proteins or proteins combined with a prosthetic group (which the organism in some cases is unable to synthesize and must obtain from its food as a vitamin). Assuming that the enzymes are produced by gene action, we are again confronted by the question whether this is a process of step-by-step synthesis dependent in each case on many genes or is one of production as a whole by a single gene presumably as at least a partial duplication of the gene pattern (Wright, 1927, 1941). The latter concept is represented diagrammatically in the middle of Fig. 2.

Sporadic examples of apparent one-to-one relation between gene and enzyme or elementary metabolic process have been known almost since the rediscovery of mendelian heredity. It has remained for the Stanford group, led by the geneticist Beadle and the biochemist Tatum, to develop a method of attacking the genetics of processes at this level in a systematic fashion in one convenient organism, the mold Neurospora. A member of the Stanford group, Dr. Horowitz, will present in this symposium some of the extraordinarily interesting results which have been obtained (*cf.* Tatum and Beadle, 1942; Beadle and Coonradt, 1944).

Another fundamental physiological process, the relations of which to genetics remain to be clarified, is growth. We must distinguish two very different possibilities for gene action. Most of the many genes, known to differentiate large and small varieties, doubtless act mainly by modifying conditions which affect the rate of growth. But growth, as the multiplication of proteins, specific to the species and individual, is a process of the same essential nature as heredity itself. Again, we have the question whether the protein molecules are produced by step-by-step synthesis by specific polyptidases (*cf.* Fruton, 1941) or are produced as wholes on a model already present. If we assume the latter and postulate that particular genes are the models for each kind of protein, we encounter the difficulty that while each gene produces only one daughter gene between cell divisions, it would probably have to produce millions of protein molecules to account for doubling of the cell. This difficulty is partially circumvented by postulating that the chromosomes contain many representatives of any gene that produces an abundant kind of protein molecule. It has, indeed, been suggested that the two kinds of chromatin that are recognized, euchromatin and heterochromatin, differ in function in that the former produces physiological agents, the enzymes, which are effective in minute amounts, while the latter produces substances needed in bulk, such as nucleic acid and tissue proteins (Caspersson, 1941a; Schultz, 1941; Darlington, 1942).

The difficulty would be further obviated, if we suppose that there are proteins in the cytoplasm that can function as models on which more proteins of the same sort can be formed. It is known that large amounts of ribonucleic acid, closely related to the desoxyribose nucleic acid found only in chromosomes, are present in rapidly dividing cells (Caspersson, 1941b). This nucleic acid is located in the microsomes and mitochondria (Lazerow, 1943; Claude, 1943). Claude has suggested that these are self-duplicating bodies.

It may be urged against the idea of such plasmagenes that there is no growth whatever in enucleate cells, but this may be due to other conditions. A more serious difficulty is the sparsity of evidence for cytoplasmic heredity (East, 1934; Correns, 1937). There is some evidence in plants but largely in relation to particular cytoplasmic constituents, the plastids. There is virtually no unambiguous evidence from higher animals. The complete transformation of species hybrids to the type of the paternal species by a few generations of backcrossing to the latter (always as male parent) in cases in both plants (Gärtner quoted by Mendel, 1866; Goodspeed and Clausen, 1917) and in animals (Stern, White and Spencer,

1944) tends to indicate that transmission outside of the nucleus is at best of casual rather than of fundamental importance.

A possible indicator of the site in the germ cells of the postulated models for protein multiplication may be obtained from the mode of inheritance of immunological specificity. The evidence from the genetics of transplantation reactions (Little and Strong, 1924) and of blood cell and serum antigens (Wiener, 1943; Irwin and Cole, 1943; Cumley, Irwin and Cole, 1943) indicates determination of each kind of specificity by a single mendelian gene. The only exceptions are the hybrid substances found by Irwin and associates in certain species crosses, and these are exceptions only in that more than one gene is involved.

One possibility of reconciling these results is the hypothesis that the cytoplasm of the egg transmits self-duplicating nucleo-proteins that lack antigenic specificity, that specificity is supplied in somatic cells by a combination with haptens produced by genes, and that this combination then behaves as a compound plasmagene (*cf.* Wright, 1941). Another (bottom of Fig. 2) is that the model nucleo-proteins are produced as wholes by duplication of special genes, and that they retain the genic property in the cytoplasm, but subject to decay, at least along the germ line. Small non-duplicating protein molecules, including enzymes, may be products of such decay. There is some evidence, not only for a process of self-duplication in the cytoplasm, but also for its decay, in the cytoplasmic lag of ciliate protozoa described by Jennings (1940) and his associates. They find gradually decreasing cytoplasmic control after a cross involving characters, that in the long run are completely determined by mendelian genes. There is more persistence (up to 36 generations) than can be accounted for by passive transmission without multiplication. The entire category of Dauermodifikationen of Jollos involving persistence for much greater periods, may come here, but the mode of inheritance is not clear.

The common denominator of these interpretations of heredity, control of metabolism and growth is dependence on specific nucleo-proteins, the genes, characterized by the property of synthesizing substances like themselves. It is possible that all gene action traces to this one process and that proteins are formed in no other way.

As in the case of growth, the problem of the nature of tissue differentiation can easily be dismissed by the geneticist as outside his conventional field. If, however, we consider the cell as an organism, a genetic problem is clearly presented in the origin of differences that persist more or less indefinitely under neutral conditions, as for example in repeated transplants of a particular kind of tumor cell.

A geneticist, on recognizing that there is a genetic problem here, naturally thinks first of the possibilities of nuclear change. There are two alternatives under this head. Nuclear differences may arise from the sorting action of non-equational mitosis, as postulated by Weismann, but now generally rejected for good reasons, or they may arise from mutations which are induced in orderly fashion under special local conditions and which thereafter stabilize an otherwise labile developmental pattern. There is evidence for a class of unstable genes in which mutation is under some degree of local control (*e.g.*, miniature-a of *Drosophila virilis* as described by Demerec, 1941) but it is a rather long step from these to orderly differentiation. Moreover, irreversible differentiation within uninucleate cells as in eggs of ctenophores and tunicates can hardly have its origin elsewhere than in the cytoplasm. The usual and more probable view is that the differences in cell heredity that arise within a multicellular organism are cytoplasmic.

Again, we may distinguish two alternatives. Persistence may be based on interactions among constituents which make the cell in each of its states of differentiation a self-regulatory system as a whole, in a sense, a single gene, at a higher level of integration than the chromosomal genes. On this view the origin of a given differentiated state of the cell is to be sought in special local conditions that favor certain chains of gene-controlled reactions which cause the array of cytoplasmic constituents to pass the threshold from the previous stable state to the given one.

There is no doubt considerable truth in this idea, but the stability of the changed state certainly becomes easier to understand if it be postulated that there are self-duplicating materials within the cell which can become modified chemically and multiply as of the new sort. The second alternative is thus controlled mutation of plasmagenes. Fig. 3 illustrates this idea in relation to a plasmagene derived from a nuclear gene and subsequently elaborated by the addition of chemical groups which become available under special local conditions. The hapten may come from outside the cell, an inductor or a hormone, for example, or as indicated in the diagram, it may be a product of a chain of gene-controlled reactions initiated under the local conditions. There could be similar gene-controlled elaboration of plasmagenes which, in their main pattern exhibit cytoplasmic heredity even along the germ line (chloroplasts, for example, in which, in maize, Rhoades (1943) has found evidence of gene-controlled mutations). Dr. Sonneborn (1943) has made some re-

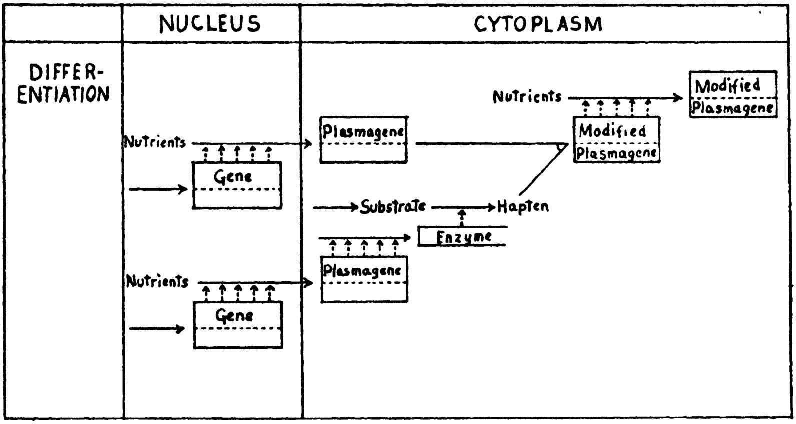

FIG. 3.

markable experiments on the relations between well-established mendelian genes of Paramecium and cytoplasmic components that are partially autonomous in the sense that, while dependent on specific genes for persistence and multiplication, they can not be produced *de novo* by these or any other genes. These results, wherever they may lead, are obviously of the greatest significance for these fundamental questions of physiological genetics. Dr. Sonneborn will discuss developments of this subject in the third paper.

The idea of differentiation of the cell as a self-regulatory system as a whole and that of stabilization of a particular mode of differentiation by means of the elaboration of self-duplicating entities within the cell are not incompatible. Something of the same sort appears at a still higher level in the multicellular organisms. We have noted already the impossibility of any one-to-one relation between gene and character at this level. In fact, each character is always affected by many genes as well as by environmental factors, and each gene in general has multiple effects. No qualitative distinction can be made between abnormalities due to gene replacement and ones due to special environmental treatment (the phenocopies of Goldschmidt, 1938). Organs and, after a mosaic phase of development, the organism as a whole, have very considerable self-regulatory power. The kinds of deviations from normal that are possible at any stage are rather limited. Any disturbance, whether genic or environmental, that surpasses the threshold of adaptability of the organism, or some part of it, calls forth one or another of these patterns of deviation. The apparent extreme localization of the effects of particular genes in certain cases turns out to be a matter of thresholds in the organism not of preformed rudiments in the germ plasm. Gene and environment are alike modifiers of a pattern residing in the whole. But the genes, while individually mere modifiers, constitute an array of physiological agents, selected through a long evolutionary history that somehow determines the self-regulatory pattern of the whole (*cf.* Wright, 1934b).

It was often asserted a few years ago that the mendelian genes determine only superficial individual differences, while the fundamental plan of development is cytoplasmic in its heredity. The demonstration that the commonest class of determinable mutations is that of lethals tends to refute this view. This was not entirely satisfactory, however, without knowledge of how these lethals act. Dr. Poulson (1940) has made very illuminating studies of such development as occurs in the eggs of Drosophila in which one or another portion of a chromosome is visibly deficient. Our last paper will deal with this important line of evidence on the genes as physiological agents.

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binations: any clone is either a resistant killer or a sensitive non-killer.

The alternative characters, killer and non-killer, are determined by a pair of allelic genes, K and k, and a cytoplasmic factor, which may be called kappa. Clones are killers only when both the dominant gene K and the cytoplasmic factor kappa are present; they are non-killers when kappa is absent, regardless of genic constitution.

The interrelations of K and kappa were investigated by studying separately the effects of each of these two factors, when removed from the other one. The effects of kappa in the absence of K were observed by obtaining self-fertilization (autogamy) within heterozygous killers (Kk plus kappa). In the resulting homozygous recessives (kk), the K genes in the nuclei have been replaced by k; but the cytoplasm is, at least at first, the same as before and so contains kappa. These recessives manifest the killer character for several fissions, then become and permanently remain non-killers. The significance of the change of phenotype in relation to kappa is indicated when gene K is reintroduced into the recessives by mating them to homozygous dominants[3] (KK). When only a few fissions intervene between the origin of the recessives from their heterozygous parents and the subsequent mating to the dominants, the reintroduction of gene K results in preventing the loss of the killer character. When more fissions intervene, the reintroduction of the killer gene is without effect: a non-killer clone develops. The gene K thus serves as a detector for the presence of kappa, and the results indicate that kappa persists in the recessives for a few fissions and then permanently disappears. Persistence of kappa in the recessives is correlated with the persistence of the killer character for a short time, indicating that kappa alone can determine the killer character in the absence of K from the nuclei. The eventual loss of kappa, however, shows that it is not independently self-multiplying in the absence of K. Gene K thus controls the maintenance (and consequent increase in amount) of kappa during reproduction by fission. This is its only known function.

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[3] In published experiments, the dominants were killers. Subsequently the experiment has been repeated with the same results using KK non-killers lacking kappa.

The fact that after gene K has been lacking from the nuclei for several fissions its reintroduction fails to restore the killer character to recessives containing cytoplasm derived exclusively from the killer race indicates not only that kappa has disappeared, but that gene K is unable to initiate the production of kappa. This is also clearly shown in much the same way by transferring K but not kappa to a pure non-killer race (kk) that possesses neither gene K nor cytoplasm of the killer type. The method is simply to cross killers (KK plus kappa) to non-killers (kk). At conjugation, each conjugant supplies the other with a gamete nucleus, but cytoplasm is normally not exchanged. Consequently, gene K is introduced into the non-killer, leaving kappa behind. The resulting heterozygote and all its vegetative and inbred sexual progeny remain permanently non-killers. Hence, both in heterozygotic and in homozygotic condition, K can not initiate the production of kappa and therefore can not determine the killer character. Nor can any other gene from the killer race initiate the production of kappa, because all of them, like K, are introduced into the non-killer at conjugation and are brought into homozygous condition in the F2.

To the non-killers containing K, obtained as just set forth, kappa may be added by conjugation with killers under certain conditions in which cytoplasm is exchanged between the mates (see experiment 1, p. 322, for details). The introduction of cytoplasm from the killer into the non-killer mate results in the production of exclusively killer progeny thereafter in both vegetative and sexual reproduction. Hence, kappa is maintained and increased in the presence of K when some kappa is present to start with.

The knowledge of kappa and K up to this point may be summarized as follows. Kappa is (1) absent from the migratory gamete nucleus; (2) present in the cytoplasm of killers at the time of fertilization; (3) essential for the development of the killer phenotype; (4) physiologically active even in the absence of gene K from the nuclei; (5) dependent upon gene K for its increase during growth and reproduction and so unable to persist after gene K is replaced by its allele k; (6) not initially producible by gene K or any other gene in the killer race. Dominant gene K (1) is unable to initiate kappa production, but (2) controls increase of kappa when kappa is already present.

II. Further Analysis of Interrelations between K and Kappa

The facts set forth in the preceding review indicate that kappa acts something like a primer in a pump: some kappa is put in and more comes out. The gene K seems to be like a pump that will not work at all without being primed: in the absence of the primer, no activity of the gene is detected. K thus appears to be a gene whose observable physiological activity—the production of kappa—is dependent upon a primer, kappa itself. This characterization of kappa as a primer of gene K is, however, merely a superficial description of a phenomenon that requires further analysis. Possibly the direct action of gene K is entirely independent of kappa. K might produce a product which has no observed effect in the absence of kappa, but which interacts with kappa to form more kappa when kappa is present. Still other and more complex interpretations are possible: for example, kappa might represent two cytoplasmic components, one independently self-multiplying and the other a primer of gene K, the two components interacting to determine the killer character. These considerations will suffice to show that the detailed relations of kappa to gene K remain to be discovered and that these relations must be known before the physiological activity of gene K can be understood.

In an effort to acquire some of the needed information, the experiments now to be set forth were performed.

Experiment 1. The relation between diverse periods of paroral union and the transfer of kappa during conjugation.

Soon after fertilization has been accomplished, conjugating mates normally separate. As a rule separation takes place last at a very small region near the cytostomes, the paroral cones, across which the migrating gamete nuclei pass during fertilization. At room temperature the great majority of conjugating pairs in all crosses (and all, or practically all, in crosses involving certain races) break apart at the paroral cones within 3½ minutes of the moment when the process of separation first appears in the anterior region. In certain crosses, however, some pairs remain united at the paroral cones for longer periods, in the extreme case remaining permanently united and eventually fusing to form a double animal. In the latter and exceptional case, cytoplasmic mixture is complete; normally, however, no cytoplasm is exchanged at conjugation. As will appear, intermediate conditions exist and, further, the amount of cytoplasmic exchange is correlated with the extent of the period of paroral union. This permits the selection and study of conjugants into which varying amounts of the cytoplasmic factor kappa have been introduced.

Crosses were made between killers (KK plus kappa) and non-killers homozygous for the killer gene (KK but lacking kappa). If cytoplasm containing kappa passes from the killer to the non-killer mate, the latter should yield killer instead of non-killer progeny (see p. 319). This test was therefore applied to pairs of conjugants which had been observed to remain united at the paroral cones for diverse periods after separating at the anterior ends.

When the period of paroral union lasted 3½ minutes or less, the non-killer conjugant never yielded any killer progeny. Hence, under these conditions, no kappa (or effectively none) passed from the killer to the non-killer conjugant. At the other extreme, when the period of paroral union lasted more than about 30 minutes, the non-killer conjugant always promptly produced a clone of killers (as in the case mentioned on p. 319). Hence, under these conditions, kappa invariably passes across from one mate to the other. Between these two extremes, when the period of paroral union lasts from 3½ to 30 minutes, a variety of results is obtained. Some of the non-killer conjugants yield exclusively non-killer progeny, some quickly develop into killer clones, and some yield clones of a type previously not encountered. Clones of this new type remain non-killers, but, unlike non-killer clones previously studied, they are capable of producing killer progeny under certain conditions to be specified later. The latter fact suggests and experiments 2 and 3 demonstrate that this new type of non-killer clone contains kappa. Further, the data indicate that the amount of kappa transferred to conjugants that produce clones of this type is less than the amount transferred to conjugants that produce killer clones, for this new type of non-killer clone arose only when the period of paroral union was intermediate between the period (less than 3½ minutes) in which no kappa is transferred and the period (more than 30 minutes) in which enough is always transferred to render the resulting clone a killer. There was, however, much overlapping of results: in some pairs with paroral unions of only 6 minutes, the non-killer conjugant produced a clone of killers; in other pairs with paroral unions as long as 9 minutes, the non-killer conjugant produced a clone of non-killers lacking kappa. Hence the duration of paroral union is only a crude index of the amount of cytoplasm and kappa transferred.

Experiment 2. Comparison of the new type of non-killer clone with its progeny produced by self-fertilization (autogamy). Proof that the new type of non-killer clone contains kappa and every other factor or substance required for the killer character.

Autogamy, or fertilization within single unmated cells, is readily induced at any time after clones are 10 to 12 fissions old. When a clone of the new type of non-killers is divided into two parts and one part is induced to undergo autogamy, while the other part is prevented from undergoing autogamy, the following results are obtained. The part that has been prevented from undergoing autogamy, but has continued to reproduce by fissions, retains the non-killer character. The part that has been permitted to undergo autogamy transforms promptly into killers. If many individuals undergoing autogamy are isolated and separately cultivated, as a rule every one of them produces a clone of killers. (The preceding results are those typically obtained when the non-killer clones are young; exceptions, further details and results with old clones are given below in experiment 4.)

Experiments 2 shows clearly that non-killer clones of the new type contain kappa and every other factor or substance required for the determination of the killer character because they are transformed into killers by means of a process (autogamy) which occurs within single cells and so excludes the introduction of material from another organism. How, then, do they differ from killers? The difference is certainly not due to a difference in genes, for the following reasons. First, the parents from which these non-killers were derived (experiment 1) were both already homozygous for the killer gene K. Second, both parents were also homozygous for all their other genes, though not necessarily for the same genes, because just a few days before the cross was made they had been through autogamy, which renders them homozygous for every gene (Sonneborn, 1942). Consequently they could yield only one genotype when crossed. The non-killers with kappa that were obtained from the cross must therefore have been genically identical with the killers obtained at the same time from the same cross. Third, when the non-killers with kappa underwent autogamy, as a rule 100 per cent. of the self-fertilized cells produced killer clones. Genic recombinations do not give that sort of result.

As non-killers that contain kappa differ from killers neither in their genes nor in the kinds of other factors or substances present, it seems that the only differences that could distinguish them are the amount of kappa they contain or the form in which it exists. As experiment 1 shows that non-killers with kappa arise only when *relatively small amounts* of kappa are introduced into KK non-killer conjugants, the failure to develop the killer character seems to be the result of this fact. If the kappa is in an inactive form in these clones, this, too, would probably be a consequence of the initial deficiency in quantity of kappa. It thus appears that the phenotype depends not only on the kinds of determiners present (K and kappa), but also on the quantity of one of them (kappa). It is remarkable that the quantity of kappa present at the time of fertilization should determine the phenotype for long periods of reproduction by fissions. To this we return later.

Experiment 3. Second proof that the new type of non-killer clone contains kappa. Cross between KK non-killers of the new type and KK non-killers known to lack kappa.

The existence of kappa in the new type of non-killers may be tested further by ascertaining whether they are capable of transferring kappa to their mates at conjugation. Crosses were therefore made between a clone of the new type of non-killers and a clone of KK non-killers known not to contain kappa (*i.e.*, one incapable of yielding killer progeny when inbred). In most of the pairs, those that separated rapidly after fertilization, one exconjugant produced a clone of killers and the other exconjugant a clone of non-killers that was incapable of yielding killer progeny at autogamy. The new type of non-killer thus transforms into a killer at conjugation as well as at autogamy. In some of the pairs, those that separated slowly after fertilization, both exconjugants produced clones of killers. Clearly non-killers of the new type had transferred to their non-killer mates the kappa required to convert them into killers. In a few pairs, some of those with intermediate periods of paroral union, one exconjugant gave a clone of killers and the other a clone of non-killers of the new type (*i.e.,* it transformed into killers whenever autogamy occurred). In other words, in these pairs also both exconjugants produced clones containing kappa and, therefore, kappa must have been transferred from one mate to the other. Hence the new type of non-killer clone contains kappa, for it not only can transform into a killer clone at fertilization, but also can transfer kappa to its mate at conjugation, thus rendering a non-killer conjugant that lacked kappa capable of producing killer progeny. This experiment also shows that the kappa in non-killers must be in the cytoplasm at the time of fertilization; otherwise it could not pass into the mate.

Experiment 4. Comparison of the effects of autogamy at different ages and in different lines of descent in clones of non-killers that contain kappa. Proof that kappa is unequally divided at fission. Evidence that this is the basis of the difference in effects of autogamy in different individuals of a clone.

Clones of non-killers that contain kappa were obtained in the way explained in experiment 1. Each of the first two, four or eight animals produced at the first fissions was isolated and separately cultivated by transfers of a single animal of each line of descent daily. The animals (usually 31 in number) left over at the time of each daily isolation were grown for less than one more day until 250 to 500 animals were present and these cultures were then tested to ascertain whether the isolation lines were killers or non-killers. Periodically, some animals from these cultures were allowed to go into autogamy, the autogamous animals were isolated and grown, and the resulting cultures were tested to ascertain whether the isolation line produced killer or non-killer progeny at autogamy. The results were different even in different lines of descent of the same clone. There were three types of lines of descent, as set forth in the following paragraphs.

(1) Very rarely, a line of descent contained no kappa at any stage of its history. Only three such lines have been found. Two of these included all the progeny of one of the first two cells produced at the first fission of an exconjugant; one included all the progeny of one of the first four cells produced at the second fission of an exconjugant. At every test these lines were non-killers and they never produced any killer progeny at autogamy. Thus kappa can be absent from one of the first two or four cells and be maintained in the other one or three cells and their progeny. Kappa is therefore occasionally divided unequally among the very first cells produced in the clone.

(2) Many lines of descent maintained kappa for 18 to 88 successive cell divisions and then lost it. These lines also remained permanently non-killers. Some of them were followed as daily isolation cultures until they died. (Clones of *P. aurelia* eventually die in the absence of fertilization (Sonneborn, 1935); in variety 4 death occurs in less than 200 fissions, usually much less.) In lines of this type, the results of autogamy differed at different stages of the life history. At any time from the origin of the clone up to a certain stage, as a rule 100 per cent. of the animals that went into autogamy yielded clones of killers. There then followed a period in which autogamy yielded lower percentages of killer clones and finally none at all. Once the latter result was obtained, autogamy never yielded killer clones at any later time. During the transition period, the cells that went through autogamy and did not transform into killers of course remained non-killers. Of the non-killers produced, the majority were incapable of giving rise to killers at any subsequent autogamy, but the remainder did yield killers at the next

autogamy. The latter, therefore, were like the parent clone: they were non-killers that nevertheless contained kappa. However, when the stage at which no killers were producible at autogamy was reached, all the non-killers were of the permanent type that did not yield killers at any later autogamy.

The existence of a transition period, in which less than 100 per cent. of the cells undergoing autogamy produce killer clones, indicates both that the amount of kappa per cell decreases gradually before it disappears and also that the percentage is a measure of the amount of kappa in the parent line. As this percentage did not always follow a steady downward course, a higher percentage of killers sometimes resulting from a later autogamy, the amount of kappa in a line of descent seems to be capable both of decreasing and of increasing during the passage of fissions, indicating again that kappa is unequally divided at fission.

The final period, in which autogamy yields only non-killer clones lacking kappa, begins in some lines as early as the 18th fission, in others as late as the 88th fission. As the capacity to produce killer progeny at autogamy is the test for the presence of kappa in the parent non-killer line, kappa disappears from these lines after having been maintained for from 18 to 88 fissions. This fact is in striking contrast to previous observations demonstrating the permanent maintenance of kappa in the presence of gene K. But previously observations were made on KK clones obtained from conjugants into which enough kappa was introduced to enable them to produce killer progeny quickly during vegetative reproduction; under such conditions, kappa is maintained indefinitely under the action of gene K. The present observations were made on KK clones obtained from conjugants into which too little kappa was introduced to enable them to produce killer progeny during vegetative reproduction; in such cases, kappa may be maintained for enormously long or for shorter periods and then disappear entirely.

(3) In addition to the two preceding types of lines, there was a third which was the most common of all. In lines of this third type kappa *never* disappeared in the course of vegetative reproduction. In some of them autogamy invariably resulted in transformation into killers: at every stage of life, every cell that went into autogamy gave rise to a clone of killers. In others, however, there occurred periods in the life history in which autogamy yielded less than 100 per cent. killer clones. At these periods the results of autogamy were the same as in the lines that lost kappa, during the period just before its loss; but in lines of this type, kappa did not disappear, and later the line again yielded 100 per cent. killer clones at autogamy. It would seem, therefore, that in these lines also the amount of kappa per cell may fluctuate from fission to fission.

During vegetative reproduction the character of lines that never lost kappa was, at least for long periods, identical with the character of the others: they remained phenotypically non-killers. Eventually, however, they seemed to change into killers, usually after 60 to 80 fissions, though occasionally after as little as 30 fissions or more than 100 fissions. Whether the observed transformation into killers actually occurred during purely vegetative reproduction is open to question for the following reason. As has been mentioned, the tests for phenotype were made, not on the daily isolation lines themselves, but on cultures grown from the animals left over at the time of the daily transfers. A technical difficulty arises from the fact that as clones grow older they become more and more apt to undergo autogamy in small mass cultures of this type, though in the isolation lines autogamy does not occur so readily and can be entirely avoided by selection (Sonneborn, 1938). As has been shown, the lines transform into killers at autogamy. Hence, if the cultures underwent autogamy before or during the test, this would make it impossible to ascertain the phenotype of the parent isolation line. The technical difficulties involved in getting a clear-cut answer to the question at issue are very great; until these difficulties are overcome, the question whether non-killers ever transform into killers during vegetative reproduction must be left open. On the other hand, it is absolutely clear that the lines under consideration retained kappa permanently: they were always capable of yielding killers at autogamy.

The three preceding diverse types of lines in the clones of non-killers that contained kappa occurred side by side within the same clone. They differ basically in only one respect: some lines lose kappa at once, some much later, others not at all. The first two types of lines described lose kappa, one immediately and the other later; the third type retains kappa permanently.

One feature common to two of the three types of lines now requires consideration. How does it happen that when the amount of kappa per cell is low in the parent non-killer line, some cells of the line yield killer clones after autogamy while at the same time some cells yield non-killer clones containing kappa and others yield non-killer clones lacking kappa? The following considerations suggest the answer. As pointed out (p. 326), autogamy was induced in the progeny of the animals left over after the daily isolation has been made (*i.e.*, among a group of about 500 animals). As shown above, kappa is unequally divided at fission and but little kappa was present in the parent line during the period under consideration. Doubtless among the many animals of any group in which autogamy was induced at that time, some had more kappa, some had less and some had none. Probably the three results of autogamy were due to its occurrence in those three kinds of animals: those with more kappa yielded killer clones; those with less, non-killer clones containing kappa; those with none, non-killer clones lacking kappa. Thus the action of autogamy could be uniform and the differences in results could be due simply to differences with respect to the kappa content of the cells in which autogamy takes place.

Certain further facts confirm this interpretation. A line of non-killers had maintained kappa for 33 fissions. At this time, instead of continuing the daily isolation line with one animal, two animals were isolated and separately cultivated. One of these yielded progeny in which kappa could never again be detected, though the first test was made on its immediate offspring; the sister cell yielded progeny which retained kappa permanently. This demonstrates how, at a critical stage, a group of only 32 animals descended from one during five fissions can include some that have and some that have not lost kappa. Had autogamy been induced in the 31 animals left over after the isolation following the 33rd fission, it would necessarily have taken place in animals differing in precisely the way postulated to account for the diversity in its effects. Thus kappa is unquestionably lost from some animals during vegetative reproduction, and autogamy in these can not restore it. Further, at times when some animals of the clone lose kappa, there must also be some animals containing intermediate amounts of kappa; and only at such times does autogamy in non-killers yield non-killers containing kappa. There can therefore be little doubt that this intermediate consequence of autogamy is due to its occurrence in animals containing intermediate amounts of kappa.

III. SUMMARY AND DISCUSSION

(1) *Summary of results.* The preceding experiments have brought to light a theoretically important fact: the existence of clones of *non-killers* that contain both gene (K) and cytoplasmic factor (kappa) hitherto thought to be sufficient for the determination of the *killer* phenotype. In previous experience, non-killer clones always lacked either kappa or both kappa and gene K. The experiments also supplied the following information about the new type of non-killer clone. First, it is genically identical with killer clones. Second, it lacks no kind of cytoplasmic factor required for the killer phenotype. Third,

it originates when the amount of kappa in a cell is low at the time of fertilization. Fourth, the kappa it contains is unequally divided at fission. This results in the loss of kappa from certain lines of descent within a clone after it has been maintained for from one to more than 88 fissions; but it also results in the permanent maintenance of kappa in most lines of descent. The question whether this unequal division of kappa at fission ever results in the transformation of non-killers into killers during vegetative reproduction is left open; but it is certain that such transformation, if it occurs, does not usually take place until after 60 to 80 successive fissions. Fifth, fertilization in the new type of non-killer clone results in the production of three types of clones—killers, non-killers with kappa, non-killers lacking kappa—and these three results seem to be determined by whether the cell undergoing fertilization has at that time more, less or no kappa respectively.

(2) *Problems.* The preceding results present four problems that must be solved in order to gain further understanding of the interrelations of K and kappa and their action in the determination of the phenotype. First, how is the difference between killers and non-killers that contain both K and kappa determined? Second, how can the unequal division of kappa at fission be accounted for? Third, how does fertilization accomplish the transformation of non-killers that contain kappa into killers? Fourth, how does it happen that the amount of kappa in a cell at the time of fertilization determines its character permanently or at least for very long periods?

(3) *Interpretations.* The experiments reported above do not provide answers to the preceding questions. However, they do provide a basis for discussing what seem to be the possible answers.

The most obvious answer that might be given is that the difference between killers and non-killers that contain kappa is due simply to a difference in the *amount* of kappa they contain. The observations in experiment 1 show that when KK non-killers receive much kappa they become killers and that non-killers containing kappa arise only when they receive less kappa. Any hypothesis must allow for this fact and must to that extent include a quantitative aspect. On the present hypothesis, the unequal division of kappa at fission would follow from its cytoplasmic localization and the lack of any precise mechanism for equal division of cytoplasm at fission. The transformation of non-killers into killers at fertilization would necessarily mean that some process or processes involved in or associated with fertilization increase the amount of kappa, though the mechanism by which this happens does not appear to be deducible from the hypothesis. To explain the increase of kappa at fertilization, additional assumptions would probably have to be made. The long or permanent persistence of the non-killer character during vegetative reproduction would imply that the increase of kappa keeps close pace with the increase in cell size and number; in other words, kappa would have to be approximately doubled during each cycle from fission to fission.

The only apparent alternative to the simple quantitative interpretation just outlined is to assume that the kappa is in an inactive form in non-killers, an active form in killers. To distinguish this from the simple quantitative hypothesis and at the same time to reconcile it with the fact that the kappa introduced into a KK cell is inactive only when small amounts are introduced, it would have to be assumed further that KK cells contain a limited amount of something that inactivates kappa without destroying it and without preventing its increase during vegetative reproduction. The inactivated form would then have to be activated in some unknown way at the time of fertilization, but never (or not for long periods) during vegetative reproduction. The inactivation hypothesis in this general form is thus more complex and therefore *a priori* less acceptable than the simple quantitative hypothesis.

On the other hand, certain general considerations, which need not be recounted here, suggested that the K genes in the macronucleus might be the inactivators of kappa, and this concrete form of the inactivation hypothesis appears to agree with and explain the results in the experiments reported in this paper better than the simple quantitative hypothesis. Unlike the latter, it explains the remarkable transformations of character at fertilization without additional assumptions. Further, it leads to predictions seemingly incompatible with the alternative hypothesis, predictions that have been confirmed by experiment. The following paragraphs attempt to set forth briefly these aspects of the special form of the inactivation hypothesis, which is perhaps better designated as the hypothesis of the combination of kappa with gene K in the macronucleus.

According to this hypothesis, the macronuclear K genes are capable of combining with kappa and thus of withdrawing it from the cytoplasm into the macronucleus. When only little kappa is available in the cytoplasm, all of it may combine with and be bound to the K genes, leaving none in the cytoplasm; when much kappa is available in the cytoplasm, the macronuclear K genes become saturated with it and leave some in the cytoplasm. These two conditions correspond to the non-killers that contain kappa and the killers, respectively. The inactivation of kappa is thus a consequence of its confinement to the macronucleus; it is active (*i.e.*, produces the killer character) only when it is also present in the cytoplasm.

As mentioned above, this hypothesis has the merit of explaining, without resorting to additional assumptions, the remarkable changes of character brought about at the time of fertilization in KK non-killers that contain kappa. The explanation is based only on the known structure and origin of the macronucleus. The adult macronucleus is known (Sonneborn, 1940) to be a compound structure consisting of at least 20 to 40 units each of which contains at least one full diploid set of genes.

This structure disintegrates at the time of fertilization and so would be expected to release into the cytoplasm whatever kappa it might contain. Concurrently two new macronuclei develop from simple diploid micronuclei; their development consists of enormous growth, accompanied by increase in the number of component units from one to the full number characteristic of the adult macronucleus. There are thus at the start only a few K genes to draw upon the kappa that had been released into the cytoplasm by the many K genes of the disintegrated old macronucleus. If many or all of the K genes of the old macronucleus had been combined with kappa, there would thus be available for the few K genes of the young new macronuclei far more kappa than they could combine with; consequently some would be left over for the cytoplasm and this would render the resulting clone a killer. If but few of the K genes in the disintegrating macronucleus had kappa, there would be only enough, or less than enough, kappa to combine with the K genes of the new macronucleus, leaving none in the cytoplasm; consequently the resulting clone would be a non-killer with kappa. Of course, if there were no kappa in the old macronucleus, there would be none in the resulting clone. The observed three kinds of results of fertilization and their relation to the amount of kappa in the cell at the time of fertilization (experiment 4) are thus explained by and are in complete agreement with this hypothesis.

The hypothesis explains equally well the observed unequal division of kappa in clones of non-killers. As mentioned in the preceding paragraph, when the amount of available kappa in the cytoplasm at the time of fertilization is very small, there would not be enough to saturate all the K genes in the two new developing macronuclei. One new macronucleus might get some kappa, the other might get none; this would result in the segregation of kappa at the first cell division, a condition that has been twice observed (experiment 4). Or the amount present might be so small that before it gets into the new macronuclei the latter have already begun to develop their compound structure, so that some units of the developing macronuclei combine with kappa and there is none left for the other units to combine with. The macronucleus divides amitotically as a whole, but the component units must undergo a sort of mitotic division except for the fact that the arrangement of the units is such as to result usually in the passage of both products of division of a unit to the same instead of to different daughter macronuclei. Consequently, if some units contain kappa and others lack it, in the course of successive fissions some entire macronuclei lacking kappa and also some that are completely saturated with kappa must arise. Here, then, is a mechanism which would assure the unequal division of kappa at fission whenever the macronucleus is not saturated with it.

The hypothesis also explains the persistence during vegetative reproduction of the non-killer character determined at the time of fertilization by the small amount of kappa present at that time. In the absence of any mechanism during vegetative reproduction by which kappa could be transferred from the macronucleus (where it is bound to gene K) to the cytoplasm (where it would have to go in order to bring about transformation of a non-killer into a killer), the unequal distribution of kappa at fission could result in only a limited range of variation in kappa: the macronucleus could become completely saturated, or, at the other extreme, kappa could be lost completely. In either case, the clone would remain a non-killer.

Finally, the hypothesis is also in agreement with the transfer of kappa from non-killers to their mates during conjugation, for the macronucleus disintegrates just prior to the exchange of gamete nuclei and so the kappa bound to K genes in the old macronucleus could be released into the cytoplasm in time to permit its passage into the mate in pairs of conjugants experiencing prolonged paroral union.

It might, however, be objected that, if the K genes of the macronucleus are combined with kappa, the K genes of the micronucleus should also be combined with kappa. If this were the case, kappa would regularly be transferred at conjugation whenever killers mate with non-killers, for the gamete nuclei are derived directly from the micronuclei. This clearly does not happen (see section I, Review); otherwise the existence of kappa as a cytoplasmic factor could never have been discovered. The micronuclear K genes are therefore not combined with kappa.. This assumed distinction between the behavior of K genes in the two types of nuclei is, however, not as incredible as at first appears, for it is well known that micronuclear genes are in general different from macronuclear genes: much evidence indicates that the former are physiologically inactive and non-essential, while the latter are physiologically active and essential.

The hypothesis of the combination of kappa with the macronuclear K genes thus agrees with and explains the observations and experimental results. What is needed, however, is an experimental test involving diverse predictions on the two alternative hypotheses. The following test seems to fulfill this requirement. The transformation of non-killers containing kappa into killers at fertilization is, on the quantitative hypothesis, due to increase of kappa by some process involved in fertilization; but on the hypothesis of combination of kappa with K, it is due to the release into the cytoplasm by the disintegrating old compound macronucleus of more kappa than can combine with the K genes of the simple newly arising macronuclei. On the latter hypothesis, if the number of newly arising macronuclei could be greatly increased, this great increase in the number of functional K genes would remove more kappa from the cytoplasm. In the extreme case, this would result in the production of a KK non-killer from a KK killer at fertilization, a result contrary to the quantitative hypothesis.

This experiment has been performed and the results agree with the expectations on the combination hypothesis: the amount of active, cytoplasmic kappa was reduced by increasing the number of new macronuclei formed at the time of fertilization. The full details of this experiment and of others along the same lines will be reported at another time. Here it need only be said that it was possible, in the way indicated, to obtain from pure killer races non-killers containing kappa and ultimately pure non-killers entirely lacking kappa.

This initial success of the combination hypothesis makes it worth while to state briefly what the hypothesis implies concerning the gene K and its relation to the cytoplasmic factor kappa. Apparently it implies that kappa, when combined with gene K, becomes in effect a part of the gene. Further, it renders intelligible both the failure of K alone to produce kappa and the priming activity of kappa. Finally it implies that the role of K as a physiological agent is essentially to duplicate itself and whatever is combined with it and to supply the cytoplasm with a product similar to itself in two respects: (1) in being able to combine with kappa and (2) in being able when so combined to catalyse the synthesis of more kappa.

In connection with certain features of this hyopthesis, some recent papers are of particular interest. Pollister and Pollister (1943) have shown that a constituent of the chromosome, the centromere, may become detached from the chromosome and exist outside of the nucleus, as a centriole, retaining its property of self-duplication. The parallel to the behavior postulated for kappa is obvious. Emerson (1944) has recently presented evidence that certain substances (antibodies) which can combine with gene products (antigens) can also combine with the gene itself. Again the parallel to the postulated or implied behavior of kappa is obvious.

Of the two alternative hypotheses presented here, the combination hypothesis seems both to fit the observations better and to lead to more interesting further developments. At present, however, the correct interpretation is still undecided. The important things are the facts themselves and the promise they hold of leading through further experimental analysis to a better understanding of what the gene is and how it works through a primer in the determination of a character.

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CHROMOSOMAL CONTROL OF EMBRYOGENESIS IN DROSOPHILA

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ANY full analysis of development and differentiation must rest ultimately on the nature of the hereditary materials, the genes, their reproduction and their activities in the physiological economy of the cell. In spite of the work directed toward the elucidation of the former two, we are in possession of very scanty direct knowledge of the genes themselves. Like other entities of science they are better known by what they do. This and other earlier symposia attest to that. Happily, the day is past when the view that genes have something to do with cellular physiology and development had to be fought for, and genes versus the cytoplasm raised the heat both of argument and of temper. The preceding papers in this series give rather full accounts of the integral place of genes in the synthetic activities of cells and the interaction between genes and primers which place us in a very advantageous position in considering the problems of development and differentiation. It is the object of this paper to present evidence obtained by approaching these problems in a different way which, together with the above, leads to the closer merging of the disciplines of genetics, embryology and biochemistry.

In the years of research on *Drosophila melanogaster*, especially since the introduction by Muller (1927) of the x-ray technique for the production of mutation, a splendid set of materials has been accumulated for the genetical analysis of developmental processes, but it is only recently that the potentialities of certain types of these materials have begun to be appreciated. The classical method has been to infer the normal activities of a gene by study of differences in effects between alleles. This suffers from the limitations of providing only partial information, and gives often a superficial or even erroneous picture of what a gene is really doing. Ideally, the best approach would be to inactivate completely, or to remove, one gene at a time and investigate the biochemical and developmental consequences. This has undoubtedly been accomplished in some, or perhaps in many, of the cases reported by Horovitz, Bonner, Mitchell, Tatum and Beadle. However, it is very difficult to distinguish cytologically any changes in the chromosomes of *Neurospora* which would indicate losses or rearrangements of genes. In *Drosophila,* on the other hand, the giant chromosomes of the salivary glands do enable us to distinguish cytological changes on a relatively fine scale. In this respect *Drosophila* has an advantage over *Neurospora,* in establishing the functions of parts of the chromosomes in relation to the standard genetic maps built up by Morgan, Bridges, Sturtevant and others.

The analysis of mutant types in *Drosophila* has proved that these are not always the result of gene mutation in the ordinary sense, but may represent deficiencies or duplications of greater or lesser portions of chromosomes. In some cases the mutant type is associated with more complex rearrangements in the chromosomes. Rearrangements without gross phenotypic manifestations are also known. But, as clearly foreshadowed by the classical research of Theodor Boveri (1902), most chromosomal deficiencies are lethal when homozygous. Conversely a large proportion of what have been called lethal genes have proved to be deficiencies. Lethal genes have always commanded the interest of the embryologist and have illuminated many developmental problems. Witness the work of Bonnevie, Chase, Chesley, Danforth, Dunn, Glücksohn-Shoenheimer, Grüneberg, Mohr and Wright on mammalian lethals. When a lethal is known to be a deficiency the simplest explanation of the lethal effects is that the loss involves a gene or genes concerned with some essential processes.

The large number of X-chromosome deficiencies and translocations in *Drosophila melanogaster* and the relative ease with which they may be handled led to the choice of the X-chromosome (Fig. 1), as the starting point for the investigation of the chromosomal control of developmental processes. By appropriate crosses it is possible to obtain zygotes which lack greater or lesser parts of a given chromosome (Fig. 2). The missing part may range in size from an entire element to less than a single band of a giant chromosome. Since these deficiencies are almost always lethal to the organism, a means of evaluating the functions of a gene in normal development is made possible by the analysis of the upsets leading to the death of the zygote carrying them. The types of embryological upsets are different in the several cases studied and make it evident that a large number of chromosomal regions are concerned in embryogenesis. In all the cases considered here the lethal effects appear in the egg stage and have been determined by examining living and fixed eggs from deficiency stocks at successive times after fertilization. This is readily done, as the larva of *Drosophila* normally hatches from the egg within twenty-four hours after fertilization.

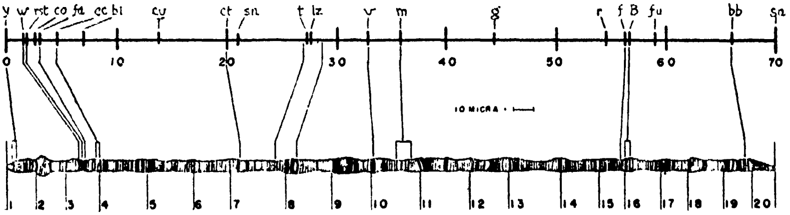

FIG. 1. Linkage and salivary gland chromosome maps of the X-chromosome of *D. melanogaster* to show the relative positions of a number of loci. Symbols of the mutants referred to in the text are: y—yellow; w—white; rst—roughest; fa—facet; sn—singed; lz—lozenge; r—rudimentary; fu—fused; sa—spindle attachment region. The numbers below the linkage map give the distances in terms of cross over units from the tip of the X. The numbers beneath the salivary gland chromosome designate the regions according to the system of Bridges. (Adapted and re-drawn after Bridges.)

NORMAL DEVELOPMENT

The sequence of embryonic development in *Drosophila*

(Fig. 3), and the higher Diptera is briefly as follows.[1] Fusion of the pronuclei takes place in the anterior portion of the egg followed by seven or eight mitoses after which the number of pre-blastoderm, or cleavage nuclei, reaches

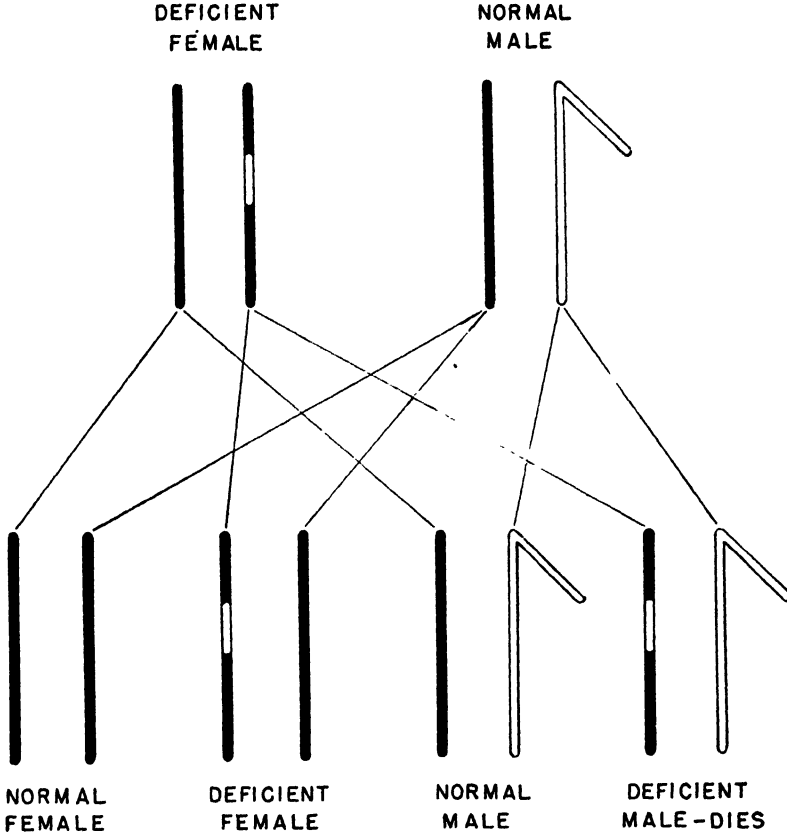

FIG. 2. The inheritance of X-chromosome deficiencies through heterozygous females. Of the four classes of zygotes only the deficient male dies at some point in development depending on the deficiency. The embryology of the others is normal.

about 256. The nuclei first become distributed more or less uniformly through the egg and are connected by cytoplasmic strands and bridges among the yolk spheres

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[1] This account is based on the work of Huettner (1923, 1924), Parks (1936), Poulson (1937, 1940a, and in press), Rabinowitz (1941), and Sonneblick (1941, and in press).

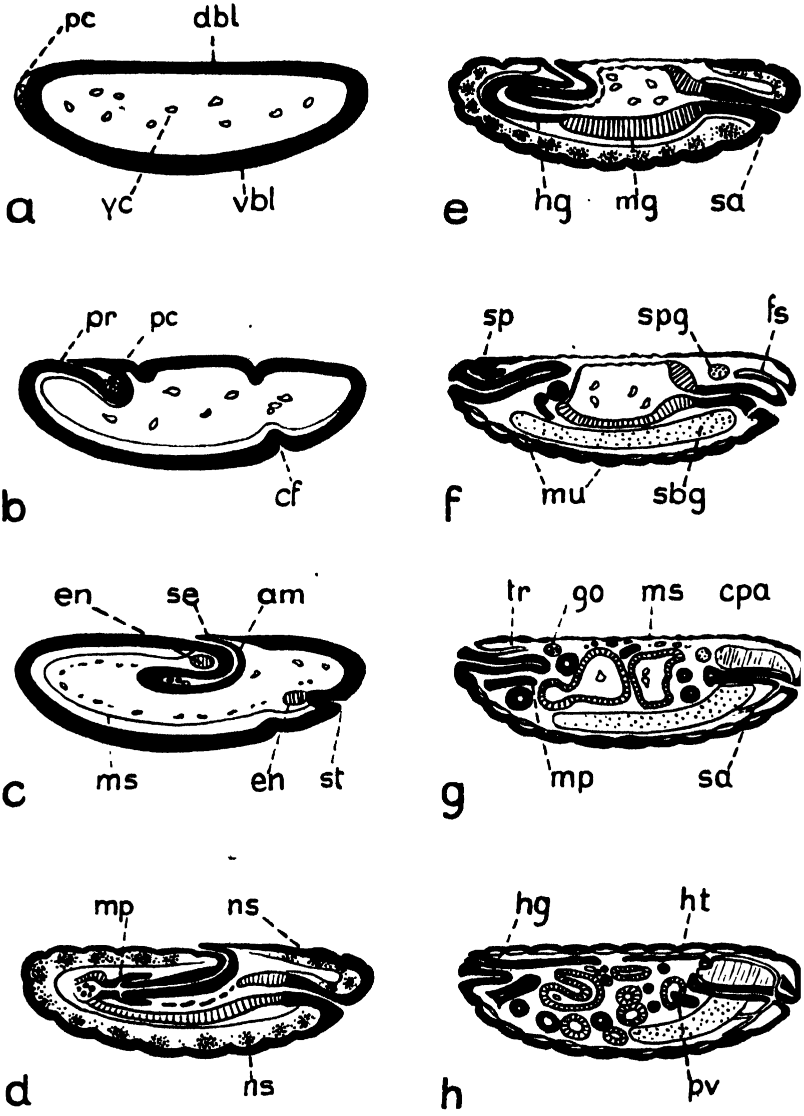

FIG. 3. Outline of morphogenesis and organogenesis in *Drosophila* from the blastoderm stage to the larva ready to hatch. Blastoderm and ectodermal derivatives shown in black, nervous system dotted; the mid-gut is cross-lined; the mesoderm unlined. a—blastoderm (2nd hr.); b—gastrulation (3rd hr.); c—extended germ band (4–6th hr.); d—later germ band showing nervous system (8th hr.); e—the contraction of the germ band (11th hr.); f—embryo following contraction (12th hr.); g—differentiation in embryo (16th hr.); h—larva before hatching (22nd hr.). Abbreviations: am—

and granules. The nuclei then begin a migration into the cortical cytoplasm of the egg. Those which reach the posterior polar plasm are differentiated almost at once to become pole cells, the future germ cells.

The nuclei which reach the cortical layer elsewhere become the blastoderm nuclei (Fig. 4a). Those which remain in the central portion of the egg become the so-called yolk or vitellophag nuclei. After several more mitoses of the blastoderm nuclei cleavage of cytoplasm between the nuclei begins and in a short time a cellular blastoderm is established surrounding the central yolk (Fig. 4d). In the third hour of development foldings in the blastoderm lead to the inturning of mid-ventral cells which constitutes gastrulation, separating outer and inner layers (Fig. 3b). Anterior and posterior portions of the latter give rise to the mid-gut and are therefore referred to as endoderm. Most of the inner layer gives rise to the mesodermal derivatives. At the posterior end of the gastrular furrow the proctodaeum is invaginated; slightly later at the anterior end, the stomodaeum. The dorsal portion of the blastoderm becomes mostly embryonic membrane. The germ band thus laid down increases in length and extends around posteriorly to the dorsal side of the egg, folding up the membranes until the proctodaeum lies just back of the head (Fig. 3c). When this extension is completed certain cells all along the ventral midline of the embryo become enlarged to form neuroblasts and move beneath the other ventral cells which will become hypoderm or other ectodermal structures (Fig. 3d). The division of neuroblasts involves unequal cytoplasmic division, the smaller products becoming ganglion cells. While this

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amnion; cf—cephalic furrow; cpa—cephalopharyngeal apparatus; dbl—dorsal blastoderm; en—endoderm rudiments of mid-gut; fs—frontal sac; go—gonad; hg—hind-gut; ht—heart or dorsal vessel; mg—mid-gut; mp—malpighian tubule; ms—mesoderm; mu—muscle; ns—nervous system; pc—pole cells or germ cells; pr—proctodaeum; pv—proventriculus; sa—salivary gland and duct; sbg—suboesophageal ganglion; se—serosa; sp—spiracle; spg—supraoesophageal ganglion or commissure; st—stomodaeum; tr—trachea; vbl—ventral blastoderm; yc—yolk cells. Only sagittal sections are shown.

process of neurogenesis is going on groups of ectodermal cells invaginate segmentally to give the tracheal pits from which the tracheal system will arise. These provide the

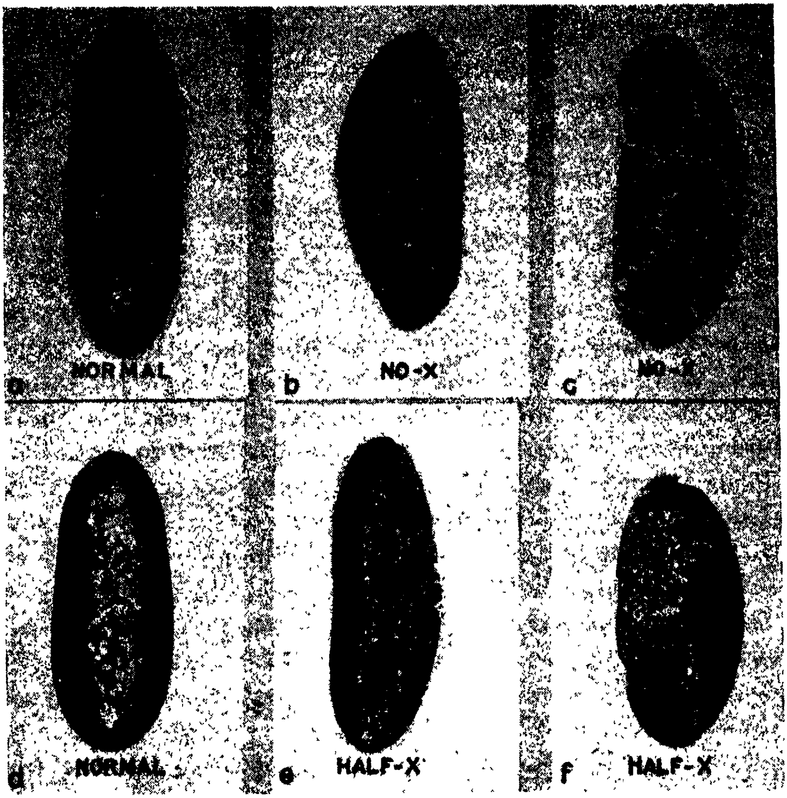

FIG. 4. Early development of normal and deficient zygotes. a.—normal embryo just before the establishment of the blastoderm; b.—nullo-X egg at the end of migration showing nuclei at periphery only in anterior half of egg; c.—nullo-X egg some hours later showing anterior mass of small cells, unincorporated cytoplasm and yolk in posterior part of egg; d.—normal embryo at the time of completion of the blastoderm with cell boundaries fully established; e.—one type of half-X deficient zygote showing incomplete and abnormal blastoderm; f.—the other type of half-X deficiency which becomes abnormal at the time of gastrulation. Photographs of longitudinal sections of iron-hematoxylin preparations. Magnification 112 ×.

first external evidences of body segmentation, except for the boundary between head and trunk which is present from gastrulation on. Anteriorly lateral groups of ectoderm cells slowly invaginate to form the salivary glands and become pulled in through the stomodaeum.

The mesoderm spreads out between the ectoderm and the developing gut and later separates into somatic and visceral portions. The two mid-gut rudiments approach each other and eventually enclose the yolk which is surrounded by the yolk cells (Fig. 3c and d). The embryo then undergoes a shortening during which the body segmentation becomes clearly apparent and the posterior end of the embryo retreats from behind the head to its more natural position (Fig. 3e and f). The lateral tissues grow out to complete the dorsal portions of the embryonic trunk.

By this time, 11 to 12 hours after fertilization, the principal processes of morphogenesis and organogenesis are completed and subsequent development consists largely of differentiation. There are no further mitoses in any purely larval tissue after this time. All subsequent growth and differentiation of larval tissues proceeds without cell division. In this period the pharyngeal apparatus, the body and visceral musculature, the dorsal vessel and fat bodies are formed, and the chitinization of skin and tracheae takes place (Fig. 3g). During this period the imaginal discs make their appearance, those of the head being distinguishable first. The fully formed larva (Fig. 3h) hatches from the egg between 20 and 24 hours after fertilization depending on the temperature.

EFFECTS OF LARGE DEFICIENCIES

The removal of an entire chromosome has long been known to be lethal, but this was clearly established for the first and fourth chromosomes of *Drosophila* by Li (1927) in his studies on the effects of chromosome rearrangements. Li did not make any developmental analysis beyond determining that mortality was in the egg stage. If zygotes lacking an entire X-chromosome are studied embryologically (these constitute one fourth of the eggs of attached-X females in *Drosophila*) developmental derangements are first evident in the cleavage stages of the egg (Poulson, 1940a). These lead to an abnormal distribution of the nuclei, which remain principally in the anterior half of the egg (Fig. 4b). Although there is cleavage of cytoplasm to form cells, no blastoderm is produced. The result, after several hours, is an anterior cell mass showing no morphogenetic advances and no differentiation. There is usually a clear stratification of the egg into cellular parts, unincorporated cytoplasm, and yolk (Fig. 4c). Yolk nuclei are frequent, but pole cells are rarely produced. The cells of the anterior mass continue division for some hours, becoming progressively smaller and evidently engaging in little or no synthetic activity. Concomitant physiological changes in nullo-X eggs are discussed below and illustrated in Fig. 8. Such drastic upsets as these, in which the whole pattern for further development is deranged, might well be expected when approximately one fifth of the egg's normal complement of genes is removed from the field of action. What will happen in cases of other deficiencies can scarcely be predicted except that, if these are large, the effects will be early and extensive. This is doubtless true for the large autosomes 2 and 3.

Among eggs produced when the X-chromosome is broken in two by a translocation near its center, T (1:4) A1, also known as the CRB translocation, are some deficient for the region between lozenge and the spindle attachment (Fig. 1) and some deficient for the remainder of the chromosome between lozenge and the left end. In each of these cases the morphological upset is drastic and early, but the two portions of this chromosome have clearly different effects. In one (the X_R deficiency ?) these are somewhat similar to the nullo-X (Fig. 4e) in that the distribution of nuclei is disturbed. This leads to the production of an incomplete blastoderm, the cells of which meet a fate much like that of those in the nullo-X eggs. In the other type of egg (X_L deficient ?) a blastoderm is formed, but separation of the germ layers fails

(Fig. 4f). Morphogenetic movements are abortive and the embryo ends up as a sac of undifferentiated cells. In both instances cell division continues for some hours, although cytological abnormalities are common.

These large deficiencies clearly show that the X-chromosome has a great deal to do with very early embryonic processes, but it is necessary to turn to smaller deficiencies to separate the effects of the individual regions and individual genes.

THE NOTCH AND WHITE DEFICIENCIES

Equally significant and no less spectacular effects have been found in instances of very much smaller deficiencies such as can be demonstrated only with the aid of the giant chromosomes of the salivary glands. Of the many deficiencies obtained in experiments by Dr. M. Demerec, of the Carnegie Institution of Washington, the most extensive series are those involving the loci of white and facet near the left end of the X-chromosome (Figs. 1 and 7). Stocks of these provided by Dr. Demerec, whose kindness is gratefully acknowledged, have made it possible to study in detail a region of the X-chromosome which controls certain very fundamental morphogenetic processes (Poulson, 1940a,b; in press). These deficiencies range in size from one removing 45 bands to those in which no visible loss is apparent. Three principal categories of deficiencies are shown in Fig. 7, those which lack both the white and facet loci, and those which lack white only or facet only.

Embryological studies clearly show that the disturbances in each of the three white deficiencies are essentially the same type. Irrespective of the size of the deficiency, organs of ectodermal origin appear to be more or less normal, but those of endodermal and mesodermal nature show clear abnormalities. These become evident between the 12th and 16th hours of development. Instead of transforming into a long convoluted tube the mid-gut remains a large undifferentiated sac without any musculature, in striking contrast to the normal condition, as in Fig. 5. This failure of differentiation of the gut is correlated with a failure of the body musculature, which begins to be laid down and then degenerates. (The same is true for aorta, fat bodies, etc.) Such embryos, while still alive at the hatching time of normal larvae, never emerge from the egg, and the yolk remains largely unutilized in the mid-gut. This is evidence that a gene or genes in the region of 3C1–3C2.3, which is that common to all three deficiencies, is concerned in the development and differentiation of mesoderm and endoderm.

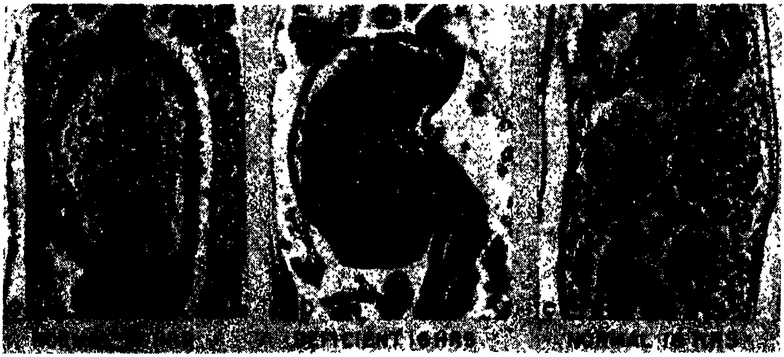

FIG. 5. The abnormal gut and mesoderm of a white-deficient embryo compared with normals. a.—frontal section of a normal embryo at 12 hours showing the mid-gut as a large sac and the beginning of the differentiation of the body musculature to the right; b.—frontal section of a deficient embryo at 16 hours showing only slight morphological advance in the gut, the absence of gut musculature and degeneration of body muscles; c.—frontal section of a normal embryo at 16 hours showing convolutions of mid-gut and presence of gut musculature. Photographs a. and c. from Bodian preparations, b. from iron-hematoxylin preparation. Magnification 272 ×.

The facet, or Notch, deficiencies, Fig. 7, all lead to an earlier and more drastic series of disturbances in which each of the germ layers is involved. The most conspicuous of these abnormalities is that far too many neuroblast cells are formed from the ventral and cephalic ectoderm so that there is little or nothing left which can give rise to skin and other ectodermal derivatives. The embryo develops a nervous system which has a total volume of cells at least three times that of normal, Fig. 6. This hypertrophied nervous system shows considerable differentiation, but the patterns of the ganglia and fiber tracts are markedly deranged (Poulson, 1943). There is no condensation such as typifies the later embryonic period in normal embryos. The nervous system remains naked and uncovered by any outer layer. Only the more lateral and dorsal parts of the hypoderm develop. Tracheal trunks are usually present dorsolaterally and are characteristically chitinized.

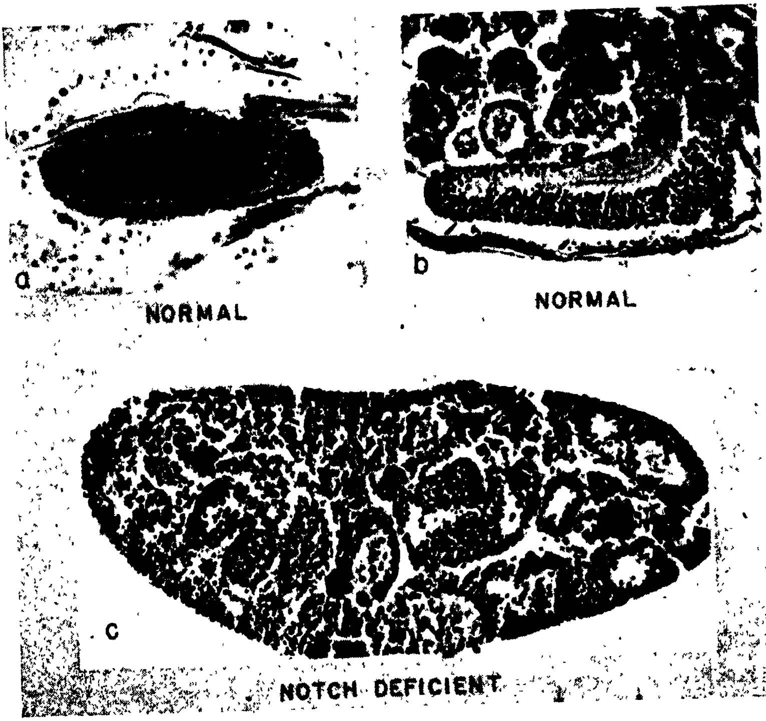

Fig. 6. Notch-deficient embryo and its nervous system compared with the nervous system of normal embryos. a.—frontal section of the highly condensed ventral nervous system of a normal embryo just before hatching; b.—sagittal section of ventral nervous system in a normal embryo of the same age as a.; c.—sagittal section of a Notch-deficient embryo showing the hypertrophied nervous system, absence of ventral hypoderm, incomplete midgut and abnormal mesoderm. Photographs from Bodian preparations. Magnification 310 ×.

The rudiments of the mid-gut fail to unite to form a proper alimentary tract, Fig. 6, and the yolk remains unenclosed by any gut. The yolk mass may occupy any of a number of positions. In Fig. 6c it is seen to lie mid-ventrally, the nervous system being bent around it. The fore-gut is rudimentary and associated structures, such as salivary glands and frontal sac, are lacking. Curiously enough the proventriculus, at the point of union of the fore- and mid-guts, is well developed. The hind-gut is more nearly normal and considerably convoluted. Malpighian tubules are usually present.

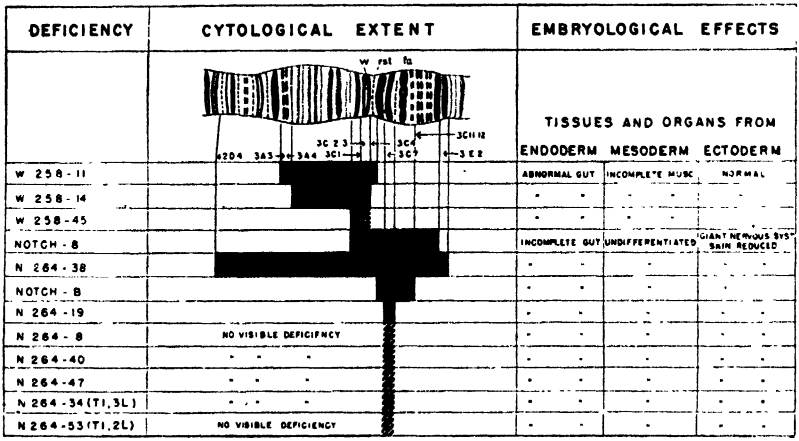

FIG. 7. Summary of the cytological extents and morphological effects of the deficiencies referred to in the text. Data on cytological extent from work of Demerec, Slizynska, Sutton, and the author summarized in Bridges and Brehme (1944). Black indicates visible deficiency; cross-lines no visible detectable deficiency, but definite genetical deficiency.

The mesoderm remains more or less undeveloped, and muscles, heart, fat-body, etc., are not produced. Partially differentiated spindle-shaped cells lying irregularly beneath the dorsal and lateral hypoderm are the nearest approach to muscles. Connective tissue is present.

All in all, a kind of hopeless monster is produced which can not develop beyond the embryonic stage, although its constituent cells and parts remain alive for some hours after normal hatching time. Since the results are the same both in the larger deficiencies and those which show nothing visibly missing, it is concluded that in all probability a single gene, which may be the normal allele of facet, is the controlling agent of the processes here deranged. The simplest hypothesis to account for the manifold effects of this gene is to assume that it is normally involved at the time of germ-layer separation and has its effects on the cells of the ventral blastoderm, or possibly only on those along the mid-line, the future mesoderm and endoderm cells. In the latter case, these, when turned under, fail to develop normally and so may influence the overlying ectoderm cells. If this is true it may mean that mesoderm normally plays a role in the induction of the insect nervous system, perhaps comparable in importance to that which it has in vertebrates. Little is known of the factors concerned in the development and differentiation of the arthropodan nervous system and the evidence from the Notch embryos may prove a step in this direction.

Embryos deficient for both white and facet (Notch–8 and N264–38) are monsters like those described above. The embryos of Notch–8 are indistinguishable from any of the other facet deficient embryos, while in the case of the largest deficiency (N264–38) the disturbance is the same, but there is even less differentiation in the various gut regions and tracheae are rarely found. Thus the gene, or genes, at the facet locus controls processes which come into operation at an earlier time than any of those controlled by genes in regions removed by the white deficiencies. Further, none of the genes or combinations of genes within the larger deficiencies appreciably modify these processes.

Two of the Notches which show no visible deficiency are associated with translocations (N264–34 and N264–53) in which the point of breakage is close to, or at, the facet locus. The available evidence in these cases suggests that deficiency for the entire tip of the X-chromosome including facet produces these same effects. Very small deficiencies near the tip of the X-chromosome investigated by Kaliss (1939) and by the writer (Poulson, 1940a) produce their effects very late in the embryo. Other deficiencies in this region reported by Demerec and Hoover (1936), Muller (1935), Sturtevant and Beadle (1936) and Sutton (1940) are the exceptional instances in which both the homozygote and hemizygote are viable and fertile. Thus it would appear that of all loci at this end of the chromosome facet influences embryogenesis earliest, and most extensively, then white and later the other loci. Other genes between bands 3E2 and 8B1 when removed with facet, 3C7, do produce an even greater dislocation of development as indicated in the X_L deficiency. The significance of these loci remains to be investigated with the aid of Notch deficiencies extending further to the right than any now available and with small deficiencies between facet and the locus of lozenge.

It is thus clearly demonstrated that different genes in the X-chromosome have quite different and often clearly separable embryogenetic effects. The individual and additive properties of most of these genes remain to be investigated step by step and related to the structure and chemistry of the chromosome.

THE PHYSIOLOGICAL EFFECTS OF DEFICIENCIES

A first step in relating the developmental effects of chromosomes to cellular physiology, in an organism whose cellular physiology is little known, is to obtain some knowledge of the overall metabolic activities in the developing zygote. Students of the physiology and biochemistry of development have been forced to make studies on large numbers of small eggs in order to have adequate material for the methods available. Provided the materials are uniform no special difficulty presents itself. When, however, there is more than one genetic type among the zygotes it becomes imperative to investigate single eggs. The perfection of the Cartesian diver ultra-microrespirometer (Boell, Needham and Rogers, 1939; Boell and Needham, 1939) provided a means of determining the respiratory metabolism of single eggs of *D. melanogaster.*

A study of the oxygen uptake of normal fertilized eggs

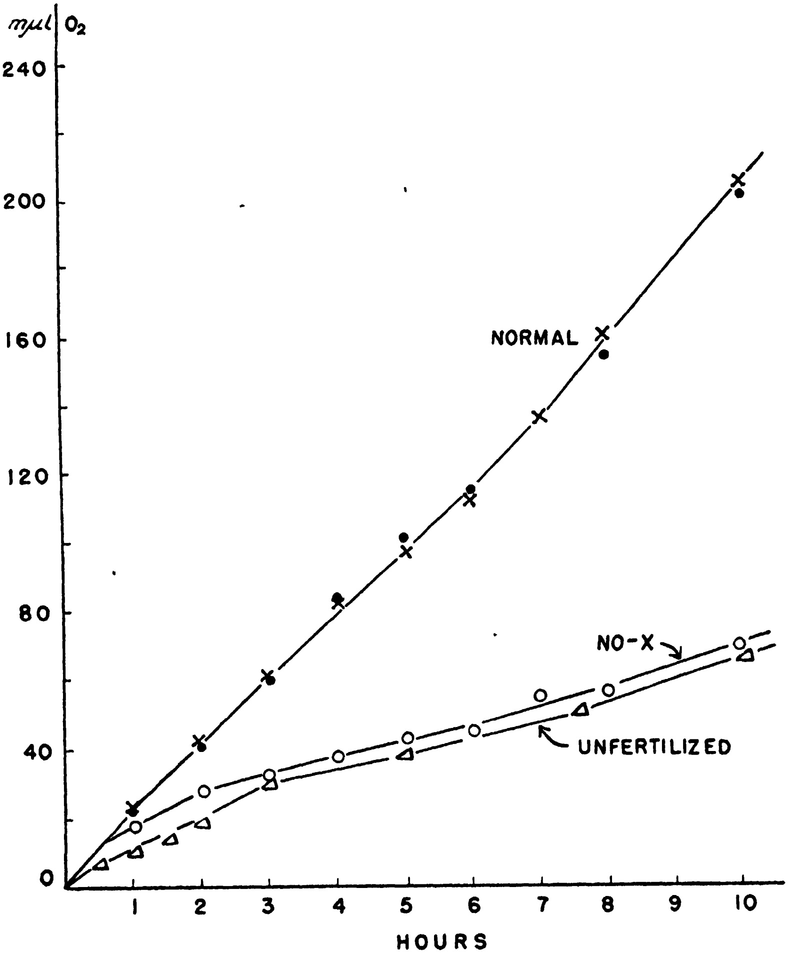

FIG. 8. The oxygen uptake of normal, unfertilized and deficient single eggs of *D. melanogaster* determined with the Cartesian diver ultramicrorespirometer. The time is in hours after the beginning of the experiment which was seldom less than an hour after eggs had been laid. The oxygen uptake is given in millimicroliters at 25° C.

of *Drosophila* (Boell and Poulson, 1939; in press) shows that the consumption is almost uniform with time, having a slight tendency to increase with age during the first twelve hours of development. The rate of oxygen uptake averages 0.026 cu. mm. per egg per hour in the Oregon R wild strain, no sex difference being distinguishable. The rates in normal non-deficient eggs of other strains are of the same magnitude, but significantly different from the Oregon R wild strain. The respiratory rate of unfertilized eggs is lower from the beginning than that of normal eggs, the final value being 0.005 cu. mm. per egg per hour.

Among eggs from attached-X stocks (of which one fourth are nullo-X), the curves for individual oxygen uptake fall into two groups (Fig. 8), that in which the oxygen uptake is as described for fertilized eggs, and one in which it is much lower. Upon microscopic examination eggs with a normal oxygen uptake have always proved to be developing normally, those with a lower rate to be either unfertilized eggs or nullo-X zygotes. In eggs deficient for the X-chromosome respiration is indistinguishable at the outset from that of normal eggs. Several hours after fertilization the oxygen consumption has declined to a value one fifth that of normal eggs. This is maintained for at least 14 hours at a steady rate which is similar to the equilibrium value of unfertilized eggs. The period in which the rate of oxygen consumption is dropping coincides in time with the appearance of the developmental abnormalities which characterize the nullo-X zygotes. The experimental data indicate that while developmental upset is accompanied by failure in respiration, the mechanism for oxygen uptake in deficient eggs is fully functional for a time immediately following fertilization. Furthermore, the cytological studies on nullo-X referred to above (Poulson, 1940a) show that cell division continues for some hours after the oxygen uptake has dropped to the final value. This raises a number of questions about the role of oxygen uptake in cell division which can not be discussed fully here (see Needham, 1942). What is clear is that cell division, though not increase in cell size or restoration of nuclear size (which means syntheses), can go on when the oxygen consumption is only one fifth that of normal eggs and of the same magnitude as in unfertilized eggs showing no signs of developmental activity. This suggests than an entire enzyme system has become inactive or has ceased to exist in the nullo-X zygotes. A comparison with the situation in other forms such as the echinoderms where Runnstrom (1930) showed that the respiration of unfertilized eggs is not sensitive to cyanide and is non-ferrous while that of fertilized eggs, proceeding at a much higher rate, is so, inevitably arises. There is also a parallel in the difference between normally developing and diapause eggs of the grasshopper elucidated by Bodine and Boell (1934). Until experiments with respiratory inhibitors have been carried out on the several types of *Drosophila* eggs we can say nothing more than that it is very probable that the difference between fertilized and unfertilized eggs which has been found is due to the inactivity or absence of the cytochrome, or Keilin-Warburg system, in the latter. The difference between normal and nullo-X zygotes is probably the same except that the system appears to be present and functional immediately following fertilization and goes out of action simultaneously with the appearance of the embryological disturbances in the deficient eggs.

Metabolic studies on the smaller deficiencies of the white-facet series have progressed only far enough to say that there are no gross respiratory differences such as just described. It seems unlikely that in these embryos in which morphogenesis and differentiation are proceeding, albeit abnormally, any large or overall effect on the respiratory metabolism would be discernible. The biochemical basis of these abnormalities will have to be approached by other methods.

INTERRELATIONS OF CHROMOSOMES AND CELLULAR PHYSIOLOGY IN DEVELOPMENT

From the embryological and physiological observations which have been presented it is clear that the chromosomes are as much concerned in the processes of development and in cellular physiology as they are in hereditary transmission. This is precisely the picture derived from the *Neurospora* studies, and both emphasize the intimate relations between the chromosomes and the enzyme systems of the cell. In *Neurospora* the correspondence between individual genes and enzymes controlling unit processes in synthesis is very close. In *Drosophila* somewhat similar, but less direct, relations between genes and enzymes are indicated, especially by the investigations on the physiology of eye pigmentation (*cf.* review by Ephrussi, 1942). In all instances the steps between the processes of cellular physiology and the visible ones of development and differentiation require elucidation.

How do genetically identical cells become differently differentiated? Relative positions and gradients (Child, 1941), by themselves, account for some differences and may result in changes in the physiology of a cell, but are not an adequate explanation for so many cases and types of highly specific differentiation. The relations between the chromosomes and units, or elements, in the cytoplasm are most certainly concerned in these cellular specializations. Wright (1941, 1945) has considered theoretically the ways in which genes and cytoplasmic units may be interrelated, and Rhoades (1943) and Sonneborn (1943) have provided specific instances in *Zea* and *Paramecium*. There are in *Drosophila* cases not unlike that which Sonneborn has presented. One is the sex-linked mutant fused (Fig. 1) studied by Lynch (1919). Homozygous fused females produce somewhat fewer eggs than heterozygotes and normals. When fertilized by the sperm of fused males (which are of normal fertility) the eggs of fused females, although they show some signs of embryogenesis, do not produce viable larvae. If fertilized by sperm from normal (not-fused) males they give rise to heterozygous females and normal (not-fused) males, but the fused males die as embryos. The nature of these developmental disturbances remains to be investigated with embryological methods. Another sex-linked mutant, rudimentary, differs from fused only in degree in this respect. Clancy and Beadle (1937) showed that the ovarian eggs of fused females and those of the mutants singed and female-sterile[2] are uninfluenced by the host when transplanted into normal females in larval stages. Thus no diffusible substance is involved within the stages investigated. The simplest hypothesis is that of non-diffusible plasma genes, or primers, controlled or synthesized by the chromogenes, or nuclear genes, and transmitted through the cytoplasm of the egg. These are exhausted before egg production in singed and female-sterile, but persist in the fused female. Introduction of the normal nuclear gene through a normal sperm into the eggs of fused females starts the synthesis again. Thus the case of fused, as well as other instances of female sterility in which eggs are produced, deserves a thorough biochemical-embryological investigation. Whether any of these mutants may be due to cytological deficiency remains to be established, but it seems unlikely in certain of the cases, such as singed, in view of the numbers of alleles some of which are normal or approach normal in fertility.

Direct evidence of a control of the cytoplasm by the chromosomes is found in the nullo-X eggs (Fig. 4), where there is a breakdown of the cortical layer at the posterior end of the egg at and following the time of migration of the nuclei (Poulson, 1940a). Thus the functional integrity of the cytoplasm depends on the presence of the nucleus in these early stages.

Since there is clear evidence in insect eggs of cytoplasmic segregation during development—widely known in the cases of the pole cells and the neuroblasts—Rhoades' and Sonneborn's suggestions concerning the role of cytoplasmic elements in differentiation apply particularly well to *Drosophila.* It is not difficult to visualize how the development of a tissue such as the mesoderm, in the white-deficient zygotes (Fig. 5), might proceed normally up to the point at which the primer normally comes into play and then cease differentiating or even de-differentiate. The hypertrophied nervous system of the facet-deficient embryos might arise through a disturbance of cytoplasmic segregation at the time of determination of neuroblasts in the ventral ectoderm, just as well as by the overzealous organizer activity suggested previously. A precise application of this point of view in the analysis of the white and facet deficiencies should prove most illuminating, but will not be attempted here because of limitations of time and space.

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[2] Eggs of singed females are abnormal in shape and size and do not develop; those of female-sterile homozygotes never become mature. The latter case may be different in nature from the others cited.

Advances in our knowledge of the chemical nature of the chromosomes (Mirsky, 1943; Mirsky and Pollister, 1942, 1943; Gulick, 1944) and of certain cytoplasmic components (Claude, 1941, 1943) demonstrate that the former consist primarily of desoxyribosenucleohistones while the latter are in large part ribosenucleoproteins. The absence of the desoxyribosenucleic acids (Mirsky and Pollister, 1943) from the cytoplasm and the presence of the ribosenucleic acid in both nucleus and cytoplasm suggests that the synthesis of the latter takes place in the nucleus under the influence of the chromosome (Caspersson and Schultz, 1940). The discovery that a desoxyribosenucleic acid is the active substance in the transformations of pneumococcal types by Avery, MacLeod and McCarty (1944) indicates that such substances possess a high degree of specificity and structural complexity. The nucleotide or nucleoprotein nature of many enzymes or coenzymes and the intimate association of some of them with cytoplasmic structures fits very well into the general picture of a system of syntheses. How other types of enzymes such as those of the cytochrome system may be related to this is an important problem.

Ordinarily we do not determine the presence or absence of an enzyme directly, but only by whether or not a reaction or a step in a reaction which it catalyzes is completed.

In both *Neurospora* and *Drosophila* the enzymes in gene controlled reactions are far from being identified. The biochemical mutants of *Neurospora* are maintained by supplying the missing products of synthesis. Whether the deficient zygotes in *Drosophila* can be made to develop if supplied with missing organic molecules or enzymes remains for the future. Even if this be accomplished there will remain unsolved the basic problem of how to pass from a knowledge of the nature of the secondary products of the chromosomes to the intimate structure of the chromosomes themselves, of integrating the isolated systems into the whole dynamic structure. It is a firm conviction of the writer that the patterns of development and differentiation of the organism are implicit in the functional architecture of the cell which in turn has its foundations in the ultimate structure of self-reproducing nucleoprotein systems, chief of which are the chromosomes. Only by filling in the patterns step by step and from level to level, while keeping clearly in mind that there are no boundaries in biological or other science—that genetics, embryology and biochemistry are all approaches to the same thing—can this difficult gap be bridged.

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REVIEWS AND COMMENTS

EDITED BY PROFESSOR CARL L. HUBBS

IN these reviews and notices of current biological publications emphasis is given to books and major articles which fall within the special scope of THE AMERICAN NATURALIST, in that they deal with the factors of organic evolution. REVIEWS AND COMMENTS are meant to include also such general discussions, reports, news items and announcements as may be of wide interest to students of evolution. Except as indicated, all items are prepared by Dr. Carl L. Hubbs, Scripps Institution of Oceanography, University of California, La Jolla, California. All opinions are those of the reviewer.

A Story Outline of Evolution. By CHARLES W. GRIMES. Boston: Bruce Humphries, Inc., 1945: i–viii, 1–244. $2.00.

THE announcement of this book rightly states that "for years the principles of evolution have stood in sore need of intelligible popular explanation." There can be no doubt that the "Story Outline" is popular in the sense of being nontechnical and unprofessional, and it is reasonably intelligent. Chief doubts arise as to whether it constitutes an adequate explanation.

Despite its deficiencies this volume will undoubtedly free many a mind from the shackles of fundamentalism, and in so doing will fulfil an obvious intent. The presentation was clearly meant to convince the religiously minded of the truth of evolution. Thus the first half of the book is devoted to a discussion of the evolution of human culture and of the machines and gadgets that go with it. Much of this is so self-evident and so generally understood as hardly to need exposition, except in that it puts the reader into the habit of thinking in developmental terms. The argument for organic evolution in the second half starts with a rebuttal of the literal interpretation of *Genesis* and even quotes William Jennings Bryan to the effect that truth must prevail, even though it may lead to the idea that man is a development of beasts. Factual statements, whether true or untrue, are seldom dimmed with any qualification, and assumptions, no matter how novel or how personal, are presented with the same force and with as little equivocation as are the supposed facts. Such tricks of writing may well enhance the effect of the book on those untrained in biology, for most people think in terms of examples, reason from analogy and seldom qualify their evidence.

The author writes with deep sincerity and great conviction, in fact with almost as much fervor as the religious bigot displays. He seems merely to have replaced an old and narrow faith with a new faith that stems from something other than a thorough understanding of nature. The lack of biological background is almost continuously evident in the discussion of organic evolution, for there is scarcely a page that does not reek with an error or a contradiction that a good biologist could have corrected. Misstatements are not infrequent in the other sections of the book as well.

Disregarding obvious contradictions, bits of long obsolete information and absurd assumptions I select as condemning evidence the following assertions: There are millions of electrons within each atom and millions of atoms within each molecule. The great glaciers moved from Alaska to the Ohio River. Bacteria are animals. The course of plant evolution is "from the algae to mosses, from mosses to ferns, from ferns to shrubs and plants and from plants and shrubs to trees." All plants reproduce through different members of the body, but in animals this process is the result of the function of different and separate bodies. There were 1,700 families of trilobites. Trilobites were the ancestors of crabs, and sea scorpions became land scorpions. The nerve conductors of the lower forms gradually wove themselves into a notochord, which in turn changed into a jointed backbone. The scales of fishes changed into the hair of mammals. Snakes paralyze their victims by a piercing glow of the eye. Milk is distilled from food. No differences in form, size or composition are apparent between the germ cells of man and of any mammal. The Chinese have an alphabet. And so on *ad nauseam*.

Preordination carried back to the Creator is the keynote. The entire course of evolution, down to the fine details, is pictured as having followed the Universal or Divine Plan of Creation. God crowded into a single cell the attributes of all plant and animal life. Plants came into being first, to add beauty and fragrance to the world and to provide also for the more corporeal needs of animals, that were to appear later. The plants "evoluted" to these ends by reason of their own intelligence, "but it is not given to our minds to understand the cunning of plant intelligence." In turn all animals arose so that, through the ordained channels, they might contribute to the origin and perfection of man.

As though such thoughts transcend the methods of evolution natural selection is scarcely considered. At times the Lamarckian viewpoint is expressed as though it were the accepted tenet of biologists. New varieties of plants have been created by cultivating and fertilizing the soil and by budding, grafting and pruning! Forelimbs developed into wings by their constant flapping! Man lost the delicate sense of smell through disuse! The work of an artist may be the result of electronic forces generated by a vision in the brain cells of a remote ancestor! (Possibly that is true of some modern art.)

A Preliminary Analysis of the Herpetofauna of Sonora. By CHARLES M. BOGERT AND JAMES A. OLIVER. Bull. Am. Mus. Nat. Hist., 83, 1945: 297–426, pls. 30–37, figs. 1–13, maps 1–2. $1.00.

To an increasing degree systematists are bringing their faunal and revisionary studies to bear on the more general problems of distribution and speciation. The paper under review is a good example of this trend. It is based on an analysis of the 125 kinds of amphibians and reptiles known from Sonora. The "herpetofauna" of this Mexican state is pictured as a mixture of "Old Northern" (pre-Holarctic) and "South American" types. From an unpublished study I can say that this is true also of the fresh-water fishes of northwestern and also of northeastern Mexico, but I would look on the "South American" elements largely as Middle American developments with a long period of endemic evolution. Routes of faunal immigration are indicated as from the south, along the coastal plain, from the Colorado Desert to the northwest, from the plateau to the east through mountain passes, and along the mountains from the south and from the north. The fishes obviously came mostly from the south by the coastal plain, to a lesser extent from the plateau through stream capture, and from the mountains to the north.

The Colorado Desert is indicated as an effective barrier and it is suggested that the greatest effect has been on older stocks with reduced adaptability. Though this great desert gives evidence of having been of long standing, the disjunct distribution of moisture-living types in Sonora is taken as evidence of a geologically recent moist period. In sharp contrast to Epling's view that the temperatures in the region were not markedly reduced in the Pleistocene, Bogert and Oliver infer from present distributional patterns that Pleistocene cold forced animals southward into the peninsulas of Baja California and Florida and the "paleopeninsula" of the Mexican plateau, there to isolate populations and thus to permit speciation. I suspect that the actual temperatures far south of the ice sheet lay between the extremes postulated by Epling and by Bogert and Oliver. It seems unlikely that entire populations were forced into Baja California by the cold, but many populations no doubt did enter the peninsula and did undergo differentiation there. Such speciation, however, may not have been solely of the peninsular category, for there is evidence indicating that much of Baja California was a large island in Pleistocene or perhaps Pliocene time.

From a herpetological basis it is concluded that exclusively areal, non-disjunct biotic provinces or regions are of limited value. "More can be gained from plotting life zones and dispersal routes than by attempts to bound biotic provinces." This criticism of a modern trend has much justice, for the biotic provinces as mapped are too generalized to be of much aid in the explanatory phases of either biogeography or speciation.

There are other data of general speciational as contrasted with herpetological interest, but these data are largely buried in the systematic account.

ARTICLES AND SHORTER BOOKS

Root Disease Fungi. A Treatise on the Epidemiology of Soil-borne Disease in Crop Plants, and a First Exposition of the Principles of Root Disease Control. By S. D. GARRETT. Annales Cryptogamici et Phytopathologici, I. Waltham, Mass.: The Chronica Botanica Co. (New York: G. E. Stechert and Co., agents), 1944: i–xvi, 1–177, figs. 1–9. $4.50.—"The root-infecting fungi, as an ecological group, afford a wealth of material to the student of evolution; to the plant pathologist, they offer a series of intricate problems in applied biology." It is obvious that the phytopathological aspects have been of most concern to the author, and it is to be hoped that he may follow through with a discussion of the evolution of these organisms. He does devote one chapter to parasitic specialization in the root-infecting fungi. The conclusion is reached that parasitism in the soil invaders arose from the saprophytic habit of the soil inhabitants. He adds that "if symbiosis be regarded as the evolutionary end-point of the parasitic habit, then the mycorrhyzal species must be regarded as the most highly specialized of all the root-infecting fungi. The parasitic habit of these plants and other phases of their ecology and physiology are then treated, as a background for the chapters on root disease control. This is an important, well-written and pioneering volume. Like other Chronica Botanica books it is neatly made.

Flora of Illinois. By GEORGE NEVILLE JONES. Notre Dame, Indiana: The University Press (American Midland Naturalist, Monograph Series, Monograph No. 2), 1945: i–vii, 1–318, 1 fig., 2 maps. $4.00.—To collect, prepare and identify 15,000 specimens, to extend this study to the previous collections deposited in such herbaria as those of the University of Illinois, the Missouri Botanical Garden and the Field Museum as well as several private herbaria, to organize and compile keys to these collections from a bibliography of over 400 listed items and to write and prepare the manuscript for publication is a colossal job on the flora of any state. That all of this can be accomplished in five years is attested by the published volume, Flora of Illinois, by George Neville Jones. With a very brief introduction of a geographic and ecological nature the main work follows in the form of synoptical keys including what there is of range, habitat, local restriction and seasonal periodicity. Brevity has been the key note. The work treats 152 families, 716 genera and 2124 species of vascular plants. The arrangement is that of Engler and Diels. Botanists will especially welcome the taxonomically classified bibliography and the originality in the glossary.—HERBERT L. MASON, Department of Botany, University of California, Berkeley, California.

Fragmenta Papuana. Observations of a Naturalist in Netherlands New Guinea. By H. J. LAM. Translated from the Dutch by Lily M. Perry. Sargentia (Arnold Arboretum of Harvard University, Jamaica Plain, Mass.), 5, 1945: i–iv, 1–196, figs. 1–32, maps A–B. $3.00.—Geographical, hydrographic, vegetational, floristic and some zoological and ethnological data are crammed into these interesting accounts of explorations in New Guinea.

Scientific Results of Cruise VII of the Carnegie during 1928–1929 under Command of Captain J. P. Ault. Oceanography—II. I. Marine Bottom Samples Collected in the Pacific Ocean by the Carnegie on Its Seventh Cruise. By ROGER R. REVELLE. II. Radium Content of Ocean-Bottom Sediments. By CHARLES S. PIGGOTT. Carnegie Inst. Wash. Publ. 556, 1944: i–x, 1–196, 14 pls., 47 figs., 12 charts. $2.00 (paper), $2.50 (cloth).

Chemistry—I. Chemical Results of the Last Cruise of the Carnegie. By HERBERT W. GRAHAM AND ERIK G. MOBERG. *Ibid.*, 562, 1944: i–vii, 1–58, 24 figs. and charts. $1.00 (paper), $1.25 (cloth).

Biology—V. The Genus Ceratium in the Pacific and North Atlantic Oceans. By HERBERT W. GRAHAM AND NATALIA BRONIKOVSKY. *Ibid.*, 565, 1944: i–vii, 1–209, 27 figs., 55 charts. $2.00 (paper), $2.50 (cloth).

FIFTEEN years after the last cruise of the *Carnegie* came to a disastrous end there are now being published some of the contributions to oceanography that resulted from this finely planned and well-executed expedition. In studying the ocean bottom samples Roger R. Revelle made use of modern methods of sedimentational and soil analysis. The objects were "(1) the determination of the various constituents of the samples with the view of ascertaining their physical and chemical nature, origin, and history before and after deposition; and (2) the classification of the samples from the point of view of the causes of the variation in bottom deposits with depth and location." The attainment of these objects will go far toward clarifying many problems in the history of the earth and in the distribution and evolution of sea life. The data, however, are very complex, and grave difficulties are encountered in arriving at objective conclusions. Factors responsible for the deposition of calcareous matter are particularly complex. The author leans toward the view that much of the firm clay on the ocean floor is of continental origin. In the short supplement Piggott reports 28 analyses of the high radium content of deep-sea deposits and reviews the few previous analyses that have been made.

In the chemical report chief attention is given to the distribution of phosphate, silica, hydrogen ions and dissolved oxygen. The relative high phosphate content of the deep Pacific and the Antarctic waters is emphasized and explained in dynamic terms. In silica content the Pacific also seems to exceed the Atlantic. In pH content the Pacific and Antarctic waters further agree and contrast with Arctic and North Atlantic waters. The very low dissolved oxygen content in the deep Pacific is taken as evidence that these waters have lacked contact with the surface layer for a greater length of time than has the deep water of the Atlantic.

In the monograph on the dinoflagellate genus *Ceratium* Graham and Bronikovsky describe regional differences in species numbers, discuss factors affecting horizontal distribution, compare the Atlantic and Pacific *Ceratium* floras, and treat the vertical distribution of these organisms. They then present extensive ecological data for each species, and supplement their account with long tables of data, many figures showing differences between and variations within species, and finally charts showing (1) the horizontal and vertical distributions of many species, (2) surface temperatures, salinities and phosphate content, (3) the clear-cut zones of species abundance, and (4) the *Ceratium* life zones.

Altogether these reports constitute a major contribution to oceanography. Their general value would have been increased by the inclusion of clear-cut interpretive summaries.

A Source-Book of Biological Names and Terms. By EDMUND C. JAEGER. Springfield, Illinois, and Baltimore: Charles C Thomas, 1944: i–xxvi, 1–256, illustr. $3.50.—This lexicon is a great improvement over *A Dictionary of Greek and Latin Combining Forms Used in Zoological Names,* by the same author, for it is much more extensive (now defining "fully 12,000" word elements), deals with botanical as well as zoological names, covers terms more thoroughly, in general gives the correct Classical roots rather than presenting combining forms with little rule or uniformity, and is more replete with interesting examples of names and terms derived from the Classical and other languages. The aim remains the same, to provide a ready means by which those who are not equipped by patience, training or library to use Classical lexicons may understand and appreciate the meaning that might otherwise lie hidden in biological names and terms. It is definitely not a manual for use in coining names, though the introduction briefly explains how words are built and quotes Palmer's interesting essay on the formation of generic names. The Greek alphabet and its equivalents and the rules for transliteration from Greek to Latin are useful features, though throughout the text the transliterating has already been done. In these days of limited or abandoned Classical training this source-book should prove useful to biological students.

Get More Out of Life! By ADRIAN J. GILARDI. Boston: Bruce Humphries, Inc., 1944: 1–192. $2.50.—With a totally non-biological view an engineer presents his thoughts on man's contact with his environment. It may be good ethics, or good medicine for sick minds.

SHORTER ARTICLES AND DISCUSSION

ISOLATING GENE E′ FOR EARLY SEXUAL MATURITY[1]

INTRODUCTION

EARLY sexual maturity is an important inherited character affecting egg production. The mode of inheritance has been studied by several investigators. Hays (1924) suggested that one sex-linked and one autosomal gene were responsible for the dominance of early sexual maturity over late sexual maturity. Waters (1934) showed that a sex-linked gene was concerned. Warren (1934) concluded that both a sex-linked and an autosomal gene must be present to produce early sexual maturity. Hays (1936), by crossing early and late maturing strains, demonstrated a cumulative effect between sex-linked gene E and autosomal gene E′. Birds carrying both genes were very early maturing, those having either genes E or E′ alone were medium early maturing, while those lacking either or both of these genes were late maturing. The phenotypes suggested were: very early, under 180 days; medium early, 180–215 days; and late, 216 days or more.

OBJECTS OF LATER STUDY

It seemed to be desirable to develop a strain of birds pure for autosomal gene E′ to discover the effect of this gene alone on age at sexual maturity and later to observe the result of bringing together genes E and E′.

EXPERIMENTAL RESULTS

Two Rhode Island Red males believed to be genetically late maturing were mated to Barred Plymouth Rock females whose age at sexual maturity ranged between 190 and 210 days. If the Barred Plymouth Rock females carried the sex-linked gene E they would transmit it only to their sons and never to their daughters. The barring gene and the silver gene would serve as markers of the sex chromosome derived from the Barred Plymouth Rock hens. Except for crossing over in the males, the gene E would remain linked with barring and silver (Warren, 1934).

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[1] Contribution 556 of the Massachusetts Agricultural Experiment Station.

CHARACTER OF F_1 DAUGHTERS

The first male gave 12 females with an age range from 179 to 215 days and a mean age of 196 days, group A. The character of the daughters indicates that their sire was a pure recessive ee e′e′ and that the Barred Rock hens were e E′E′ because all daughters were medium maturing.

The second male gave 12 daughters with an age range between 177 and 312 days and a mean age of 211 days, group B. Their phenotypes with respect to age were 1 early; 9 medium; 2 late. This ratio suggests that the sire carried the sex-linked gene E and that the dams were heterozygous for gene E′.

CHARACTER OF THE F_2 DAUGHTERS

To produce the F_2 generation an F_1 male from group B was mated to F_1 daughters from group A all of which showed medium maturity. A total of 16 F_2 daughters was produced from this mating. Four of these daughters exhibited barring and silver, indicating that their single sex chromosome had passed unaltered from their Barred Rock granddam. All four of these were definitely late in sexual maturity, having a mean age of 297 days. This indicates that sex-linked gene E was not present in the original Barred Rock females. Since these females were all phenotypically medium in sexual maturity they must have carried autosomal gene E′.

In the F_2 generation four daughters appeared that carried a crossover sex chromosome with silver and lacking barring. Two of these were medium in maturity and two were late. Only one daughter with barring and lacking silver was tested for age at sexual maturity and she matured at 215 days.

Seven daughters carrying their Rhode Island Red grandsire's sex chromosome were tested. These daughters lacked both barring and silver. Two out of the seven showed medium maturity and five were late maturing. If their grandsire had carried gene E, no less than one half of these females would have shown the effects of gene E and extra early sexual maturity would have appeared in some daughters because of combined effects of genes E and E′.

A second group of F_2 females was produced by mating an F_1 male from the group described in the preceding paragraph to F_1 females descending from a Rhode Island Red male that carried sex-linked gene E mated to Barred Rock females that were heterozygous for autosomal gene E′. The F_1 daughters tested showed 1 very early to 9 medium early to 2 late maturing. Two of these late-maturing females were mated to the F_1 male. Two daughters were tested and one carrying barring and silver as contributed by the sex chromosomes of the grandmother showed medium maturity because of gene E′. The other showed late maturity. From the second mating to a late maturing F_1 female two daughters carried early maturity. One carried the original sex chromosome of the grandmother BS and the other inherited her sex chromosome from her grandsire bs. There were two late maturing daughters each with a bs sex chromosome.

Three medium maturing F_1 females from this same group were mated to the F_1 male. A total of seven daughters was tested for age at sexual maturity. The result was 5 medium to 2 late. Since no very early maturing daughters appeared it is probable that both the sire and dams lacked gene E and were heterozygous for gene E′. This would give a 3 to 1 ratio which was closely approached. Only one medium maturing daughter carried barring and silver. One of the late maturing daughters carried barring and silver and the other was a crossover with silver alone.

CHARACTER OF THE THIRD GENERATION DAUGHTERS

An F_2 male from group B females was mated to five phenotypically late-maturing Rhode Island Red females with an age range from 257 to 298 days. This F_2 male was of a medium light shade of Rhode Island Red color and carried neither barring nor silver. It is very probable that neither of his sex chromosomes came from his Barred Rock grandmother. He evidently carried only gene E′ for early sexual maturity. From this mating appeared 11 medium early daughters and 11 late daughters exactly as expected. The mean age of the medium early daughter carrying gene E′ was 200 days and the mean of the late daughters was 307 days.

A supposedly late-maturing Rhode Island Red male was mated to two F_2 females that lacked barring and silver. One dam U715 matured at 254 days and gave when mated to the Rhode Island Red male 3 medium (mean 199 days) daughters to 4 late (mean 252 days). This same male mated to U1115 maturing at 203 days gave two medium to two late daughters. The Rhode Island Red male selected must have carried gene E′ because he gave equal numbers of medium and late daughters from a late dam and because he gave no very early maturing daughters from a dam transmitting gene E′.

CHARACTER OF THE FOURTH GENERATION DAUGHTERS

One solid black male lacking barring and silver and hatched in the F_2 generation was mated to five females produced in the third generation. These females were sired by an F_2 male mated to late Rhode Island Red females. Two of these females were phenotypically medium early in maturity (200 and 210 days, respectively). From the first of these hens all eight daughters were phenotypically late, but this appeared to be due to pathological conditions. The second female gave 8 medium early (mean 201 days) and 3 late (mean 249 days). The expectation would be $\frac{3}{4}$ medium $\frac{1}{4}$ late if both parents were heterozygous for gene E′.

This same black male was also mated to three phenotypically late-maturing females (338, 307 and 334 days). Nine daughters were tested of which four were medium (211 days) and five were late (273 days). This ratio suggests that the male carried gene E′ in heterozygous condition.

CHARACTER OF FIFTH GENERATION DAUGHTERS

A male of the fourth generation was mated to four of his full sisters to make a generation essentially homozygous for gene E′. All sisters selected showed medium early sexual maturity. From these matings 14 daughters were tested and all showed medium early maturity with a mean age of 213 days. The mean of 213 days is somewhat greater than previous means for females carrying gene E′ and the increase in age may be due to inbreeding (Hays, 1924).

A male of the fourth generation from a late maturing dam was chosen and mated to a group of his full and half sisters. So few progeny resulted that no daughters were tested for age at sexual maturity.

CHARACTERS OF THE SIXTH GENERATION DAUGHTERS

A male was selected from a family of Rhode Island Reds that carried genes E and E′. The family of 19 sisters to this male had an age range from 156 to 203 days with a mean of 178 days.

This Rhode Island Red male was mated to two very early maturing Rhode Island Red females (163 and 166 days) from which daughters were tested. A total of six daughters was tested and they showed a mean age of 184 days. The sire was evidently Ee E′E′ or Ee E′e′ in genetic composition and the two dams were probably EoE′E′ because they gave 3 very early to 3 medium early daughters as would be expected from such a mating. The mean age of the very early daughters was 175 days and for the medium early the mean was 192 days.

This Rhode Island Red male was also mated to three medium hybrid hens (212, 215 and 203 days). These hens carried only gene E′ for which they were homozygous. The mean age of the five daughters tested was 182 days. There were 2 very early to 3 medium early daughters, as expected. The mean age of the very early daughters was 172 days and of the medium early 190 days. There is thus very excellent agreement in results from mating one male to two kinds of hens.

A hybrid male known to carry gene E′ alone was mated to two very early maturing Rhode Island Red females (168 and 156 days). Four daughters tested from these matings had a mean age of 181 and all were medium early maturing as expected.

This hybrid male was also mated to two medium maturing Rhode Island Red females (206 and 208 days). Five daughters were tested from these matings and their mean age was 198 days and all were medium early.

SUMMARY

By marking the sex chromosome with such dominant genes as barring and silver it is a relatively simple matter to eliminate the sex-linked gene E for early sexual maturity in hybrids between Barred Plymouth Rocks and Rhode Island Reds. When the sex-linked gene is eliminated it is not difficult to develop a strain homozygous for autosomal gene E′ alone. This procedure has been followed and a line pure for gene E′ was later crossed with a line carrying both genes E and E′.

It was observed that the mean age at sexual maturity of groups of daughters carrying gene E′ alone usually ranged from 190 to 200 days under the conditions of the experiment. When genes E and E′ were brought together the mean age was reduced to from 170 to 175 days. When both genes were absent the mean age at sexual maturity was variable with means of groups of daughters ranging from 250 to 300 days.

F. A. HAYS

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SEX DETERMINATION IN TRITURUS VULGARIS LINN (TAENIATUS SCHNEID)

THE genetics of sex determination in Urodela are far from understood.

Humphrey (1942, a, b) converted a genetically female individual of *Amblystoma mexicanum* into a male by a testis implant which was later removed. With normal females it gave about 3 females to 1 male, and while about two-thirds of these females gave a normal sex ratio with normal males, one-third gave all female progeny. The 3:1 ratio was also observed among the offspring of two genetically female *A. tigrinum* similarly changed into males. Humphrey concludes that the male is XX, the normal female XY, and the thelytokous female YY. If so, the X and Y chromosomes can be little differentiated, femaleness behaving as if due to a gene which is completely dominant, at least in diploids. Such a simple sex-determining mechanism is clearly less stable than one in which the YY homozygotes are inviable, and we should expect to find different mechanisms in related species, as in the Cyprinodont fishes.

Fankhauser (1940) found that a triploid *Triturus viridescens* male was normal, while females had poorly developed gonads. This was confirmed by Griffiths (1941). Fankhauser concluded that the males are XX or XXX, females XY and the undeveloped triploid females XXY.

But in *Triturus pyrrhogaster* the triploids described by Kaylor (1943) were all females with well-developed gonads, suggesting that the female is here homogametic. The comparable evidence regarding the mechanism of sex determination in *Triturus vulgaris* so far rests on two animals, an adult functional triploid male found by Book (1940) in Sweden and a haploid female described by Fankhauser (1938), and obtained experimentally while working in Switzerland. These are explicable if the male is X, XX or XXX, the females Y, XY or YY, as would be expected if the mechanism were the same as in *Amblystoma mexicanum*.

There are however, two further pieces of circumstantial evidence concerning *Triturus vulgaris*. Pariser (1932) by means of artificial fertilization obtained offspring from the cross *T. vulgaris* ♀ × *T. cristatus* ♂. Of these 121 contained ovaries, 1 contained testes and in 1 the gonads were undifferentiated. A great preponderance of one sex is commonly observed among species hybrids and very few exceptions have been found to "Haldane's rule" (1922) that the sex which is deficient is the heterogametic sex. This therefore strongly suggests that in both *T. vulgaris* and *T. cristatus* the female is normally XX.

Van Swinderen (1928) found neotenic members of *T. vulgaris* in 7 ponds in Western Holland, the females being fertile, but the males sterile till after metamorphosis. In one of these ponds a number of the neotenic females were white with black eyes, resembling white axolotls. The gills were bright red, and the tails so transparent that their vertebrae could be counted. No pigment was visible macroscopically. No intermediates were found.

Van Swinderen suggested that the whiteness was due to endocrine disturbances. However, every sharp color variation in part of a population that has been investigated has been found to be gene controlled. With the exception of black-and-white in Ayrshire cattle (Wentworth, 1916) which has different dominance relations to red-and-white in the two sexes, every such variation in tetrapods found only in one sex is due to sex-linked genes, though the action of color genes in *Lebistes reticulatus* is limited to the male. However, a naturalist who found a rare variation confined to females in a bird population would provisionally ascribe it to a sex-linked recessive gene.

Therefore at first sight van Swinderen's observation supports the theory that the female in *T. vulgaris* is XY. However, normal sex linkage is only possible where there is a differential segment between the X and Y chromosomes, *i.e.*, where the Y carries no allelomorph of the locus concerned. But in this species a haploid female (on this hypothesis without an X chromosome) has been fairly viable and was not white. Therefore the differential segment must be small and it is unlikely that it exists at all.

Another explanation of van Swinderen's population can be made by comparing it with the polymorphism of the beetle *Phytodecta variabilis* (de Zulueta, 1925) and the segregation of several *bobbed* mutant allelomorphs in about 3 per cent. of females in many wild populations of *Drosophila hydei* (Spencer, 1938). These animals show a sexual dimorphism just because both X and Y chromosomes carry the loci concerned.

These loci extremely rarely, if ever, undergo recombination with the sex-determining parts of the chromosomes. Hence a recessive mutation on the X chromosome can give rise to recessive zygotes of the homogametic sex only. Recessives can only occur in the heterogametic sex as the result of independent mutation in the Y chromosome, or of crossing over between the X and Y, if this occurs. A recessive mutation on the Y chromosome can only give rise to recessives in the normally heterogametic sex if it meets a corresponding allele in the X, or if a YY or Y zygote is viable, and is formed as a result of sex reversal or some other abnormal process.

On this hypothesis the appearance of white females is explained by the female *T. vulgaris* being homogametic, and the mutant gene causing whiteness and neoteny having arisen on the X chromosome near enough to the sex-determining gene or region to prevent recombination between the date of the original mutation and of the inbreeding that caused the segregation.

Thus evidence is presented supporting the suggestion that in *T. vulgaris* as in *T. pyrrhogaster* the female is the homogametic sex. If this is so Fankhauser's haploid female would be X and Book's triploid male most likely XYY, though as the sex-determining mechanism is variable within the genus it may even vary within races of the same species (compare Winge's experimental results with *Lebistes*, 1934). Reciprocal interspecific crosses between two species or races which had different homogametic sexes would be expected to produce different sex ratios and degrees of differentiation. The results might be expected to be complicated, as the chromosome pair differentiated into X and Y might not be homologous in the two organisms.

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SOME OBSERVATIONS ON LEAF SHAPE EXPRESSION IN THE MALVACEAE

DEVELOPMENTAL studies of leaf shape in *Gossypium* (Stephens, 1944; in press (c)) have shown that all leaf shapes in the genus pass through, or are potentially capable of passing through, three successive phases: (1) an entire (unlobed) leaf phase; (2) a phase in which the leaves at successive nodes become progressively laciniated into five lobes; (3) a climax phase in which leaf shape is stabilized. This development is controlled by a single multiple allelomorph system. Monogenic control of leaf-shape patterns of a similar kind is found in other genera in the Malvaceae, *e.g.*, in *Malva* (Kristofferson, 1923), in *Hibiscus cannabinus* L. (Deshpande, 1942), and recently evidence of a similar system has been obtained in *Hibiscus rosa-sinensis* L. which will be discussed here.

An examination of the different horticultural strains of *H. rosa-sinensis* grown in Trinidad shows that in most cases the climax leaves are entire. In certain types, however, when the seedlings are pruned back hard enough to encourage dormant buds to develop towards the base of the stem, it is noticed that the first leaves to unfold are lobed. Later, the normal entire leaf is regained. Most ornamental strains of *rosa-sinensis* are highly sterile, but at least two, "Hawaiian White" and "Apricot," set very occasional capsules by open pollination. Seeds from a single capsule of the latter were sown out in order to observe the juvenile leaf stages. Eight seedlings were raised, of which three were entire leaved throughout their life history. In the remaining five plants, the first leaves were entire, but subsequent leaves became progressively more laciniated until about the tenth node. Later, secondary lobes were developed which progressively obliterated the main sinuses and at the flowering stage most of the leaves had become secondarily entire. These plants could be further classified into two types in the juvenile stages, *viz.*, intermediate and narrow, according to the maximum laciniation achieved (see Fig. 1). The family as a whole segregated as follows,

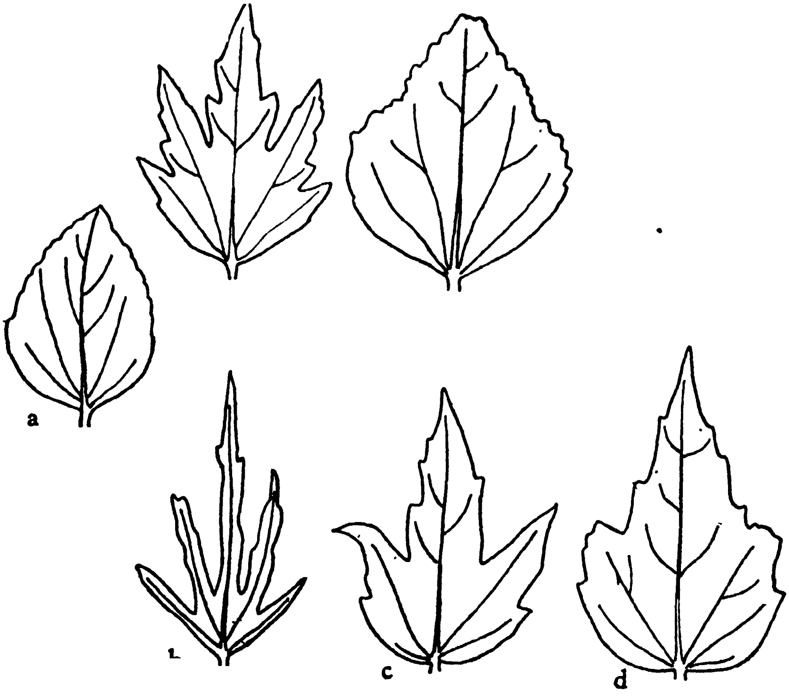

FIG. 1. A comparison of leaf-shape development in "intermediate" (top row) and "narrow" (bottom row) types of *Hibiscus rosa-sinensis*. Primary stage (a); successive stages—juvenile to climax leaf (b), (c) and (d). In a third type the primary stage is retained throughout the life history (see text).

$$3 \text{ entire} : \underbrace{3 \text{ intermediate} : 2 \text{ narrow}}_{2^\circ \text{ entire}}$$

which is possibly an approximation to the 1 : 2 : 1 ratio expected on selfing a mono-hybrid. Since "Apricot" is known to be a secondarily entire leaved type, and as Deshpande has shown that monofactorial leaf shape inheritance occurs in the genus, this is probably the correct interpretation.

The developmental behavior of these leaves is of particular interest since it makes possible a comparison, which transcends the limits of a single genus, between similar developmental mechanisms. In the development of the *Hibiscus* and *Gossypium* leaves, the first three phases are clearly similar, but in the former a fourth phase supervenes which has no true counterpart in the latter. However, in the *Gossypium* system there is one allele (L^R) which produces a climax leaf with accessory lobes, *i.e.*, a five-lobed leaf with an extra lobule on either side of the median lobe (Stephens, 1944). It is possible that given a suitable genotypic background, the action of the L^R allele could be accelerated so as to produce a continuation of development in which the accessory lobes would eventually obliterate the main sinuses (as actually occurs in the *Hibiscus* system). Such a genotypic background is probably not available in *Gossypium*, but it is known that the rate at which the leaf-shape alleles in this genus work is greatly modified by altering the flowering habit (Stephens, in press (b) and (c)). It appears, for instance, that on the late-flowering, perennial background provided by the arboreal species, *G. aridum* (R and S) Skovsted, lobing is retarded and the leaf remains in the primary entire leaf phase. The converse effect, *i.e.*, acceleration of development in leaves already lobed, which would be of more interest in the present instance, is much less easily demonstrated since the breeding of early flowering annual types for commerce has already set a limit to further acceleration. Nevertheless, considerable evidence is available, from developmental observations of species whose leaf shapes have not yet been studied genetically, which supports the thesis of a basic leaf shape mechanism common throughout the Malvaceae. In *Hibiscus esculentus* L. leaf-shape development is of the *Gossypium* type—not of the type found in its sister species, *Hibiscus rosa-sinensis*, and at least two of the climax leaf shape patterns which occur have recognizable counterparts in the cultivated cottons. The leaf-shape patterns of the related genera, *Thespesia, Shantzia* and *Gossypioides,* can also be matched by patterns occurring in American diploid and Asiatic species of *Gossypium* (Fig. 2).

It can scarcely be doubted, then, that similar monogenic mechanisms occurring throughout one genus (*Gossypium*) and also in related genera (*Hibiscus* and *Malva*) implies an original homology which has been retained through their diverging evolutionary histories. If this is accepted the possibility must also be admitted that the phenotypic expression of a gene may only represent a fraction of its full potentialities, that is, quite apart from considerations of mutability and segregation. It is well known that variability in genetic expression at a single locus does not depend solely on mutation and segregation but may be conditioned by "modification," *i.e.*, by change of balance with genes at other loci. Usually, however, modification is considered a minor source of variability and it is not so often realized that its potentialities may be enormously greater than is commonly observed within the limits of a single species. Were it not for the isolating mechanisms which restrict interspecific, and usually prevent intergeneric recombinations, it seems likely that a greatly extended range of modification could be achieved, resulting in the expression of many otherwise latent genetic characters. In the present example it appears likely that the potentiality for producing a secondarily entire leaf, latent in *Gossypium,* is expressed in *Hibiscus,* and it can not be assumed that the final expression has been reached in either genus if it is reached at all! The phenotype provides, as it were, only a cross-section of the genotypic structure, and in each section only a limited range of the individual genes' activities can be observed. Furthermore, it is important to realize that the range of expression can be shifted considerably without altering the genotypic background.

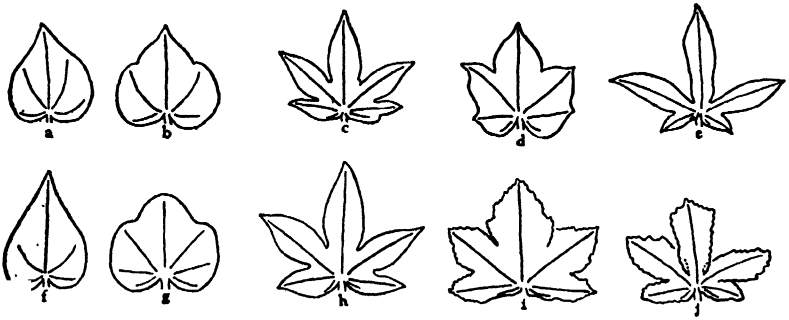

FIG. 2. Comparison of various *Gossypium* leaf shapes (top row) with their phenocopies in related genera (bottom row). (a) *G. aridum* (R and S) Skovsted; (b) *G. harknessii* Brandg.; (c) *G. arboreum* L. (L^1 allele); (d) *G. hirsutum* var. *taitense* Hutchinson (1 ellele); (e) *G. hirsutum* L. (L^O allele); (f) *Thespesia populnea* Soland; (g) *Shantzia garckeana* Lewton; (h) *Gossypioides bussei* Hutchinson; (i) and (j) *Hibiscus esculentus* L. Leaves reduced to approximately equal sizes for purpose of illustration.

In *Gossypium*, alterations in the photoperiod may exert a considerable effect on the rate of action of the leaf-shape alleles (Stephens, in press (a)) and this provides the clue to the striking leaf-shape modifications observed in *Cannabis sativa* L. by Schaffner (1926). The recent work of Went (1944) suggests that thermoperiodicity may be a still more potent factor in shifting the range of phenotypic expression. A new environment may therefore reveal hidden potentialities, without in the first instance shifting the genotypic balance by natural selection. This "plasticity" is already familiar to experimental taxonomists (Turesson, 1922; Clausen *et al.*, 1940)—its genetic basis is clarified by a developmental approach.

The peculiar course of leaf-shape development in *H. rosa-sinensis,* where an entire leaf becomes laciniated only to return subsequently to the entire condition, suggests a possible explanation of the dissected cotyledons found in certain wild species of *Gossypium,* notably *G. sturtii* F. Muell. Haldane (1932) has drawn attention to the striking possibilities of changing phenotypic expression by altering the timing of gene action. Arguing along similar lines, one might suppose that acceleration of leaf-shape development in *Hibiscus* would throw back the laciniated phase earlier and earlier in the ontogeny of the plant, eliminating eventually the primary entire phase and initiating laciniation in the cotyledons. This situation would resemble superficially that actually occurring in *G. sturtii,* where laciniated cotyledons are succeeded by entire foliage leaves.

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MINIMUM INTENSITIES OF ILLUMINATION UNDER WHICH OWLS CAN FIND DEAD PREY BY SIGHT

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Introduction

It is well known that owls are able to find their way about and to secure prey at night in very weak light. Few previous attempts, however, seem to have been made to determine the precise lower limit of an owl's vision.

In the experiments here reported an effort has been made to determine the weakest amount of illumination in which individuals of four species of owls are able to find the carcasses of freshly killed deer-mice (*Peromyscus maniculatus*). All the species of owls tested are known to feed in nature on Peromyscus and all of our captive owls fed readily on either live or dead mice belonging to this species.

The laborious daily work of conducting the experiments was performed by E. C. Sensenig, W. E. Howard, Elizabeth Barto, Mary Jane Lagler, and more briefly, by several other persons.

Methods

The reaction room in which the experiments were performed is located in the Vertebrate Biology Building of the University of Michigan. It measures approximately nine by twenty-one feet in inside dimensions. There is a single door near one end of the room. A partition twenty-four inches high extends across the middle of the room and divides it into two compartments, each nine by ten and one-half feet in size. The corners of each compartment are rounded by means of sheet-metal shields. Ventilation for the room is provided by an electric fan. There are light traps for the incoming and for the outgoing air. Special care has been taken to make the reaction room light-tight, but in spite of all precautions there is a slight amount of light leakage into the room, especially around the blocked-off windows and around the door. However, the amount of entering light is very slight, and one fails to see any trace of light until he has been in the darkened room for a number of minutes.

A perch for the owl extends across the room directly above the middle partition. It is about six feet above the floor. At one end of this perch is a large box to which the owl retires when the mice are being placed in position. The box has a door that swings open when its catch is released by pulling a string which extends outside the reaction room.

Illumination for the reaction room is provided by electric-light bulbs held in fixtures mounted on the ceiling over the middle of each of the two compartments. The lights are controlled from a panel outside the room. The controls include a rheostat and a time clock. The lamp fixture used for obtaining the weaker light intensities consists of a half-gallon tin can which completely surrounds a light bulb and which has an opening only on the lower end. The effective opening is 3½ inches in diameter and is covered by a variable number of round sheets of paper. By increasing the number of sheets of paper the intensity of the illumination produced by the lamp may be reduced. The same type and thickness of paper, No. 3 University linen, was used in all the experiments. As many as sixteen sheets of this paper were used to reduce the illumination to the lowest intensities employed. The Mazda lamp bulbs used in this fixture were of two types: 10-watt daylight and 6-watt inside blue. When the resistance provided by the rheostat was introduced into the circuit the light produced by either of these bulbs was reduced to approximately one-third its normal value. Surrounding this special fixture was a cluster of three light sockets, each with a large white enameled reflector. In these sockets there were used 50-watt daylight Mazda, 150-watt daylight Mazda, and No. 2 photoflood bulbs, as desired.

The hue of the light at the various intensities employed was not exactly the same. The light produced by the 6-watt inside-blue bulb is slightly more violet to our eyes than that produced by the 10-watt daylight bulb. An increase in the number of sheets of paper placed under the lamp bulb causes an apparently slight increase in blue tone. On the other hand, the reduction of light intensity by use of the rheostat makes the light appear less blue. No simple way of measuring these variations in hue has been found. It is believed, however, that the range of these variations in color is not very great. The over-all hue produced by the lamps appears to our eyes to be not very different at the several intensities used, being slightly more blue than daylight. The photoflood bulbs gave light somewhat richer in yellow and red than daylight, but these bulbs were used very little.

The measurement of the light intensities falling on the floor of the reaction room has been made by a special Weston illumination meter, model 603. The instrument is provided with two light-sensitive cells and measures the range from 0.02 to 2.00 foot-candles. This meter is able to measure directly only a small part of the range of intensities used in these studies. The values of the lower intensities were calculated by a proportional method. One of the cells of the photometer was covered and the other placed a few inches below the paper-covered opening of the ceiling lamp. The reading of the photometer could then be translated into light intensities falling on the floor by computation, using the rule that the decrease of light is proportional to the square of the distance. The proportional difference in light intensity produced by the several types of electric-light bulbs employed was also determined, as was the effect on the light intensity of inserting the rheostat resistance. The reduction of light produced by additional sheets of paper in the lamp fixture was also measured, using the stronger bulb. By a series of calculations it was then possible to compute roughly the light intensity at the very dim illuminations.

No claim is made that the light intensities at the very weak illuminations used has been determined accurately. Numerous sources of error are involved in the calculations. The original readings of the photometer were of necessity rather rough, and any errors made at that time were of course multiplied in calculating the lower light intensities. The photometer, although calibrated for artificial light, was not accurately adjusted to the spectrum given by each of the several lamp bulbs employed. This would especially be true when the rheostat had reduced the bulb temperature and when the light had passed through a number of sheets of white paper in the lamp fixture. It is known, furthermore, that the voltage of the power line supplying the lamps was subject to considerable fluctuation. The intensity of light produced by each bulb undoubtedly varied also with its age. Although the amount of light leakage into the room was small it probably contributed an appreciable amount to the general illumination at the lower light intensities employed. The slight amount of diffuse light reflected from the walls and floor of the reaction room also could not be estimated. The calculations of light intensity should therefore be considered to be only rough approximations.

The procedure of each experiment was to cover the floor of one compartment in the reaction room with soil of a light color, the other compartment with a soil of a dark color. Two colors of mice were also employed, one type roughly matching the color of the lighter soil, the other that of the darker soil. One mouse of each color was killed and then, while the owl was in his box, the bodies of the two mice were placed in a more or less natural position on the floor, both usually in the same compartment. The positions of the mice on the floor of the compartment were usually changed for each experiment, so that the owl could not form the habit of searching in a particular place. In general, the mice were placed alternately on successive days in each of the two compartments. After the bodies of the mice were in position the door of the reaction room was closed, the illumination of the room adjusted to the proper intensity, and the owl released by pulling the string that opened the door of his box.

The owl was then allowed to search for the mice overnight or for a given interval of time, after which he was made to retire to his box and the mice which remained were counted. A mouse found by the owl and either eaten or moved by him to a new position was counted as a capture, whether or not the mouse was eaten immediately.

After the experiments for the day were completed the owl was given additional mice for food, but these were removed after they had been exposed for a few hours, so that at the time for beginning the experiment on the following day the owl was always hungry.

In the attempt to keep the conditions as natural as possible for the owl the reaction room was left dark about twelve hours of each twenty-four and was illuminated by six 150-watt Mazda daylight bulbs during the other twelve hours. These lights were controlled by a time clock. The periods of light and dark were, in some of the experiments, purposely set out of step with the dark and light periods of normal night and day, so that the time each day when the experiments were begun would approximate in diurnal time the coming of darkness and the beginning of usual owl activity.

The soils used in the experiments were of two types, here called, respectively, silica sand and gulley soil. The silica sand, which is a regular article of commerce, i whitish in color. The gulley soil was secured from a small eroded gulley in some filled-in land alongside the laboratory building. This soil evidently contained very little humus. It was a dull, slightly yellowish gray in color.

The dead mice employed were of three types: albino, ivory, and gray. All of them were laboratory strains of the deer-mouse (*Peromyscus maniculatus*). The ivory mice were a close match in color with the silica sand. The albino mice were more nearly white than the silica sand and therefore they contrasted with it slightly in shade. The gray mice were a mixed lot of animals discarded from time to time from the laboratory stock. A few of them matched the gulley soil fairly well in color, but some were different from this soil in hue and many of them were considerably darker in shade.

RESULTS OF THE EXPERIMENTS

The experiments here described were seriously begun in the fall of 1938, when the special reaction room above described was constructed for the purpose. Tentative experiments had been initiated a number of years earlier, but had to be given up because of the lack of a satisfactory place for the study. All the experiments here reported were completed before the end of 1943.

BARRED OWL

The barred owl (*Strix varia*) used in these studies was an adult female presented by Hugh S. Davis, who secured it at Tulsa, Oklahoma. Its subspecies has not been determined.

In fairly bright light the barred owl has no difficulty in seeing mice at a distance of ten feet or more. Several times while we were watching her the owl pounced from her perch onto live mice which were running about on the floor of the room. Her method was to drop on the floor near the mouse, throw her wings more or less around the animal, and seize it in one of her talons. The maneuver was carried out very rapidly and it was difficult to observe the precise details. At light intensities of 8.5, 0.4, and 0.3

foot-candles the owl evidently distinguished the mouse clearly and attacked it directly. These intensities correspond, respectively, to weak daylight and to the light given by the full moon overhead on a very clear night. There is nothing to indicate that this owl would not be able to see well in still brighter light.

In dim lights the movements of the owl could not be clearly seen by the human observer. By noting the tracks of the owl in the sand on the floor of the reaction room, however, an indication was secured whether the owl was able to pounce directly upon or near a dead mouse or whether she approached it by stalking. With light from a 6-watt inside-blue bulb, reduced by the rheostat, and passed through nine sheets of paper (producing about 0.000,000,73 foot-candle of illumination on the floor) the owl was able to pounce directly upon or near a dead mouse, and did so repeatedly, as shown by her tracks at the position where the mouse was placed.

When the light was dimmed by ten sheets of paper (giving about 0.000,000,53 foot-candle of illumination) the owl was still sometimes able to drop directly onto the position of a dead mouse. Out of ten mice exposed during five nights on silica sand at this light intensity two albino mice were pounced upon directly, four albino and two gray mice were secured by extensive searching on foot, and two albino mice were not found by the owl.

With eleven sheets of paper (0.000,000,35 foot-candle) and at still weaker light intensities, with one exception, the owl was never able to pounce directly onto the dead mouse, but always approached it on foot. In the one exception noted the tracks in the sand indicated that the owl had dropped directly onto a dead mouse in complete darkness. On this occasion, however, a dead mouse had been placed in exactly the same position for several successive nights and it seems probable therefore that the owl had developed a position habit. In subsequent trials the positions of the dead mice were changed each night, and the owl then was not able to secure any mice in the dark.

At some of the lower light intensities employed the barred owl evidently was able to see the mouse when she was close to it, though she could not pounce directly upon it from a distance. Often her tracks in the sand seemed to be aimless until she came within a foot or so of a mouse, whereupon she went directly to it. This type of behavior was particularly noticeable when eleven or twelve sheets of paper were used to reduce the light to calculated intensities of 0.000,000,35 or 0.000,000,25 foot-candle, respectively. With eleven sheets of paper, however, the owl on one occasion passed within six inches of a mouse without finding it.

At the lowest light intensities employed and in darkness the barred owl usually was unable to find the dead mice, even though she was allowed to search for the whole night. In the morning the floor of the reaction room would show many tracks, especially in the vicinity of places where mice had been secured on previous nights.

In order to exhibit more precisely the lower limit of illumination at which the barred owl could find the dead mice the most extensive series of experiments performed is summarized in Table I. In these experiments two dead mice were exposed to the owl each night.

It will be noted from the table that the owl was able to secure mice at somewhat lower light intensities on the nearly white silica sand than on the darker-colored gulley soil. Thus, the owl secured no mice on the gulley soil under illuminations below 0.000,000,19 foot-candle, but she secured five mice out of eight exposed on silica sand at an illumination of 0.000,000,15 foot-candle. The reflection of light from the pale-colored silica sand evidently slightly increased the effective light intensity in that compartment.

Also, it will be noted that mice contrasting in color with the background on which they were placed were taken at somewhat lower light intensities than those that were of a color more nearly matching that of the soil. No albino mice were taken on silica sand under an illumination of

TABLE I

BARRED OWL. NUMBER OF DEAD DEER-MICE FOUND IN LIGHT OF VARIOUS INTENSITIES AND IN DARKNESS. TWO MICE EXPOSED TO ONE OWL OVERNIGHT IN EACH TRIAL

Illumination in foot-candles	Silica sand				Gulley soil				Mice taken per night Average
	Albino mice		Gray mice		Albino mice		Gray mice		
	Number exposed	Per cent. taken	Number exposed	Per cent. taken	Number exposed	Per cent. taken	Number exposed	Per cent. taken	
0.000,001,05 ...	..	..	..	...	1	100	5	80	1.7
0.000,000,73	1	0	1	100	2	100	6	67	1.4
0.000,000,53	3	67	7	100	5	60	5	20	1.3
0.000,000,35	3	67	7	100	4	100	6	17	1.4
0.000,000,25	4	50	4	100	3	100	5	20	1.3
0.000,000,19	8	25	15	67	4	50	9	0	.8
0.000,000,15	3	0	5	100	5	0	3	0	.6
0.000,000,11	1	0	5	20	..	...	..	..	.3
0.000,000,08	..	..	6	0	..	...	..	..	.0
Dark	..	..	10	10	..	...	..	..	.1

less than 0.000,000,19 foot-candle, but gray mice were secured on this color of soil under light intensities of both 0.000,000,15 and 0.000,000,11 foot-candle. Likewise, on the gulley soil no gray mice were taken at an illumination less than 0.000,000,25 foot-candle, but several albino mice were secured by the owl at an illumination of only 0.000,000,19 foot-candle. These data are in harmony with the general observation that objects contrasting in color with their background are more easily perceived than those that harmonize with it.

It is evident then that in stating the minimum light intensity at which an owl can see its prey the color of the soil and of the prey must both be specified.

An inspection of the table will reveal that for any given color of mouse and of soil there is no exact light intensity at which the owl suddenly fails to find prey. On the contrary, there is in general a gradual dropping off in the percentage of prey secured as the illumination becomes dimmer. It is not easy to determine the precise lower limit of vision of the owl, because she secured an occasional mouse in extremely dim light and in darkness, obviously by searching on the floor of the room until she came into contact with a carcass.

The barred owl seemed to have no great difficulty in finding gray-colored mice on silica-sand soil at a light intensity calculated to be 0.000,000,15 foot-candle. These are the most favorable conditions for vision. At this very dim illumination she was not able to find albino mice on silica-sand soil, nor any mice at all on the darker-colored gulley soil. At an illumination of 0.000,000,11 foot-candle the owl secured only one mouse in five trials, this one probably by chance.

The failure of the owl to secure all the mice exposed at the higher light intensities does not demonstrate any lack of vision at those illuminations. Often the owl secured one mouse during the night and did not search any more, perhaps because she was no longer hungry.

These experiments demonstrate that the barred owl is able to see in extremely dim light. At a light intensity calculated to be 0.000,000,73 foot-candle the bird certainly was able to see mouse carcasses of various colors on silica-sand soil at a distance of six feet or more. This is proved by her ability to pounce from her perch directly onto the mice. At a light intensity calculated to be 0.000,000,53 foot-candle she was only occasionally able to pounce directly onto a carcass, but found her prey by searching on foot. It seems almost certain that she is able to see a mouse carcass in light of this intensity when she is within a foot or less of it. By searching on the floor of the reaction room the owl was able to find dark-colored mice with considerable frequency on nearly white soil in light intensities calculated to be as low as 0.000,000,15 foot-candle. In such dim light the owl never found the mouse carcasses that were not contrasting in color with the soil nor did she find any carcasses on dark-colored soil. There is no evidence from these experiments that this particular barred owl was able to see anything at all in light intensities below 0.000,000,15 foot-candle.

Long-eared Owl

The long-eared owl (*Asio wilsonianus*) used in these experiments was captured in Pennsylvania and was kindly presented to us by the Pennsylvania Game Commission through Dr. Richard Gerstell. The bird was evidently adult when received by us. It is still alive and its sex has not been determined.

This long-eared owl regularly captured living and dead deer-mice exposed in light of moderate to strong intensities. A series of experiments designed to determine the lowest limit of light intensity in which the owl could secure Peromyscus carcasses is summarized in Table II. In these experiments one dead deer-mouse of ivory color and one of gray color was exposed to the owl each night.

In light passed through eight sheets of paper (0.000,001,05 foot-candle calculated) the owl made few tracks in the sand on the floor of the reaction room and seemed

TABLE 11

LONG-EARED OWL. NUMBER OF DEAD DEER-MICE FOUND IN LIGHT OF VARIOUS INTENSITIES AND IN DARKNESS. TWO MICE EXPOSED TO ONE OWL OVERNIGHT IN EACH TRIAL

Illumination in foot-candles	Silica sand				Gulley soil				Mice taken per night Average
	Ivory mice		Gray mice		Ivory mice		Gray mice		
	Number exposed	Per cent. taken	Number exposed	Per cent. taken	Number exposed	Per cent. taken	Number exposed	Per cent. taken	
0.000,001,05	8	75	8	75	8	88	8	88	1.63
0.000,000,25	8	25	8	88	8	100	8	13	1.13
0.000,000,08	8	25	8	38	8	38	8	13	.56
Dark	8	0	8	0	8	25	8	25	.25

to have no difficulty in locating the mice directly from his perch. When twelve sheets of paper were used, reducing the light intensity to 0.000,000,25 foot-candle, the owl evidently was not able to see well at a distance and he made many tracks in the sand in searching for the dead mice. In one trial at this light intensity, the owl actually walked over a gray-colored mouse lying on gulley soil and moved the carcass slightly in position without securing it. At still weaker light intensities the owl made many tracks on the floor of the reaction room. He secured a few mouse carcasses, however, even in complete darkness. It may be doubted that the owl was able to see anything at all in the slight amount of light passed through sixteen sheets of paper (0.000,000,08 foot-candle), and it is believed that the mouse carcasses secured at this light intensity and in complete darkness were secured only by the owl accidentally coming into contact with them in his meanderings on the floor of the reaction room.

These data fail to prove any greater ability of this owl to secure mouse carcasses of one color or on one color of soil than another at any light intensity. However, the number of records secured at the lower light intensities is few.

So far as may be told from these rather meager experiments the long-eared owl is able to see from a distance of six feet or more and to pounce directly upon prey when the light intensity is 0.000,001,05 foot-candle. The lower limit of illumination at which the long-eared owl is able to see dead prey close at hand is indicated to lie between 0.000,000,25 and 0.000,000,08 foot-candle.

BARN OWL

The barn owl (*Tyto alba pratincola*) used in these experiments was presented by John Craighead, who had captured it in southern Michigan. It is still alive and its sex is unknown.

In most of the experiments with the barn owl the bird was allowed only a 20-minute period in which to secure his prey, instead of a whole night (Table III). These experiments were performed in the morning after the owl had been exposed to light of approximately nine foot-candles intensity for the preceding night, in the attempt to make him reverse his usual diurnal cycle of activity. If the owl failed to secure a mouse in the first 20-minute trial period of the day, he was given additional test periods, each counting as a trial. Each successive trial was, however, carried out at a light intensity somewhat different, usually stronger, than the preceding one. The owl thus was sometimes given two or three, or very rarely four, trials in one morning.

Under an illumination calculated to have been 0.000,002 foot-candle the barn owl secured six mice in ten trial periods and it seems evident that he could see very well in this amount of illumination. At a light intensity estimated to be 0.000,000,53 foot-candle he secured one of the two dead mice placed in the room in three trials out of ten. In this dim light he, therefore, undoubtedly had some difficulty in seeing the mice. At a dimmer illumination estimated to be 0.000,000,31 foot-candle he secured only one mouse in ten trials. At a still weaker light intensity, estimated to be 0.000,000,08 foot-candle, the owl failed to secure any mice. Some of the mice taken in the weaker light intensities were undoubtedly located by the owl through random searching in the dark, but this could hardly account for his being successful in securing three mice in ten trials at the intensity of 0.000,000,53 foot-candle. In complete darkness the owl secured only one mouse in twenty trials.

It will be noted from the table that the barn owl failed to take any mice in ten trials under an illumination of 0.000,013 foot-candle. Also at several other light intensities he was successful only part of the time, although he later demonstrated his ability to secure mice in considerably weaker light. The explanation for these failures seems to be that this owl was very timid and that he had not become fully accustomed to his surroundings at the

TABLE III

BARN OWL. NUMBER OF DEAD DEER-MICE FOUND IN LIGHT OF VARIOUS INTENSITIES AND IN DARKNESS. TWO MICE EXPOSED TO ONE OWL FOR 20-MINUTE PERIODS

Illumination in foot-candles	Silica sand				Gulley soil				Mice taken per period Average
	Ivory mice		Gray mice		Ivory mice		Gray mice		
	Number exposed	Per cent. taken	Number exposed	Per cent. taken	Number exposed	Per cent. taken	Number exposed	Per cent. taken	
8.5	5	0	5	40	5	0	5	20	.6
1.58	5	40	5	40	5	20	5	50	1.5
.24	5	10	5	30	5	10	5	20	.7
.000,1	5	0	5	10	5	0	5	10	.2
.000,026	5	0	5	40	5	0	5	20	.6
.000,013	5	0	5	0	5	0	5	0	.0
.000,003,5	5	10	5	10	5	0	5	0	.2
.000,002	5	10	5	30	5	10	5	10	.6
.000,000,53	5	10	5	10	5	10	5	0	.3
.000,000,31	5	0	5	10	5	0	5	0	.1
.000,000,08	5	0	5	0	5	0	5	0	.0
Dark	10	0	10	0	10	0	10	10	.05

time when these early experiments were carried out. There seems to be no doubt that the barn owl can detect and secure mice over a great range of light intensities, for he demonstrated an ability to see in very weak illuminations as well as in fairly strong ones.

This owl exhibited, early in the tests, a decided preference for the gray over the ivory mice offered. Efforts to break him of this preference were not entirely successful, for the total number of gray mice (36) taken considerably exceeds that of the ivory mice (13).

In these experiments, as in those with the barred owl, there is a tendency under weak illumination for more mice to be secured on the nearly white silica sand than on the gray gulley soil. This difference is assumed to be due to the greater amount of light reflected from the lighter colored soil, which presumably increases the total illumination in that compartment to a slight extent.

In a few experiments the barn owl was given the whole night to search for mouse carcasses on the floor of the reaction room. In these experiments two dead gray mice were placed each night on silica sand, to which they contrasted conspicuously in shade of color. For five nights the light intensity was adjusted to an amount calculated to have been 0.000,000,08 foot-candle. Under this amount of illumination the barn owl secured both dead mice on each of three nights and one mouse on one night. On the other night the owl evidently did not leave his perch, for he secured no mice and there were no tracks in the sand. For five other nights, alternating with the first set, the reaction room was in complete darkness. Nevertheless, the owl secured both mice on two nights and one mouse on each of two other nights. On one night he secured no mouse, though he searched diligently, as shown by the numerous tracks he made in the sand.

Every morning, except two, during the period this later series of experiments was being conducted the sand on the floor of the reaction room was found to be covered by tracks of the owl. On one night, as above mentioned, no tracks were made on the floor and the owl failed to secure a mouse. On the other exceptional night the owl secured one mouse in complete darkness, but there were tracks only within a foot or two of the place where the mouse was secured. In this instance the bird may possibly have found his prey shortly after his first descent to the floor and then did not search farther that night.

It seems evident, therefore, that under very weak illumination and in complete darkness the barn owl secured his prey mostly by walking about on the floor of the reaction room until he came into contact with a carcass.

The fact that the barn owl was somewhat more successful than the barred owl or long-eared owl in securing mice in very weak illuminations and in darkness during an over-night period seems to be due to his greater energy in searching. Although both of these other species of owls did search for the mice on the floor of the reaction room, as shown by their tracks in the sand, neither of them made as many tracks as did the barn owl.

These experiments demonstrate that under these laboratory conditions the barn owl undoubtedly is able to see dead mice six or more feet away under an illumination calculated to be 0.000,002 foot-candle, but that he has some difficulty in seeing the carcasses under the most favorable conditions when the incident light is reduced to 0.000,000,53 foot-candle. Under illuminations dimmer than this the owl probably secured his prey almost entirely by searching on the ground on foot.

Burrowing Owl

The western burrowing owls (*Speotyto cunicularia hypugaea*) used in this study consisted of two females from Alamogordo, New Mexico, secured from H. G. Clark. This owl is considerably more diurnal in habit than the other species used in this investigation. In their natural habitat, burrowing owls are frequently seen in early morning or late afternoon perched on fence posts or other points of vantage. It, therefore, is of considerable interest to discover whether they are able to see their prey in such dim illuminations as the more nocturnal, forest-inhabiting kinds of owls.

Both burrowing owls ran about incessantly over the floor of the reaction room, much more so than did the other species of owls tested. It seems evident that much of their prey must be secured by being run down, rather than swooped upon. Every night the floor of the experimental room would be completely covered by the tracks of the two owls. This habit made difficult the exact determination of the lower limit of illumination under which the owls could see. It was impossible to be certain that a dead mouse taken during an experiment actually was located by sight rather than stumbled upon in the course of the meanderings of the owls. To make the finding of the mice more difficult for the owls they were given only a 20-minute period in which to secure their prey, instead of a whole night.

At the highest light intensities which were used in the experimental room (109 foot-candles) the owls were able to locate the mice satisfactorily and there was no indication that this amount of light was the least handicap to them. Together they secured an average of 1.3 mice in each of nine 20-minute periods (Table IV). From their habits it seems probable that they often feed in full sunlight.

In light intensities down to and including 0.000,026 foot-candle the owls secured an average of about one dead mouse on each 20-minute trial. At light intensities lower than this they were generally unsuccessful. At an intensity of 0.000,013 foot-candle the two owls together found only one mouse in ten trials, and at 0.000,003,5 foot-candle only two mice in ten trials. In complete darkness they were able to find one mouse in ten trials. It seems certain that the mice secured at the lower light intensities, like those found in darkness, were located by chance, rather than by sight.

When the owls were given the whole night to search for

TABLE IV

BURROWING OWL. NUMBER OF DEAD DEER-MICE FOUND IN LIGHT OF VARIOUS INTENSITIES AND IN DARKNESS. TWO MICE EXPOSED TO TWO OWLS FOR 20-MINUTE PERIODS

Illumination in foot candles	Silica Sand				Gulley Soil				Mice taken per period Average
	Ivory mice		Gray mice		Ivory mice		Gray mice		
	Number exposed	Per cent. taken	Number exposed	Per cent. taken	Number exposed	Per cent. taken	Number exposed	Per cent. taken	
109	4	50	4	0	5	60	5	40	1.3
8.5	5	20	5	40	5	80	5	0	.7
1.58	5	60	5	40	5	60	5	40	1.0
0.24	5	60	5	80	5	60	5	40	1.2
0.017	5	100	5	20	5	100	5	20	1.2
0.004,5	5	60	5	80	5	100	5	0	1.2
0.000,1	5	0	5	40	5	60	5	0	.5
0.000,026	5	40	5	60	5	80	5	20	1.0
0.000,013	5	0	5	20	5	0	5	0	.1
0.000,003,5	5	0	5	20	5	20	5	0	.2
Dark	6	17	6	0	5	0	5	0	.1

the dead mice they were more successful than in the 20-minute periods. At a light intensity calculated to be 0.000,001,05 foot-candle they secured six mice in ten nights. The owls, however, also found six mice in ten nights in complete darkness. It therefore seems certain that these mice must have been secured mostly by chance encounters rather than by the use of vision.

The evidence from these experiments indicates that the lower limit of effective vision of the burrowing owl is reached somewhere between a light intensity of 0.000,026 and 0.000,013 foot-candle.

NOCTURNAL ILLUMINATION IN NATURAL HABITATS

The light from the zenithal full moon that falls on the earth on a clear night may be taken as being 0.02 foot-candle. The photometer that I used gave approximately this reading on a bright moonlight night in November, 1939. At quarter-moon the light intensity is reduced to 0.002 foot-candle. Starlight is said to have an intensity of 0.000,08 foot-candle (Smithsonian Institution, 1918: 218).

The light falling on the earth on a moonless night is, however, increased by zodiacal light and by a variable amount of auroral luminosity in the earth's atmosphere. I am informed by Dr. W. C. Rufus, of the University of Michigan Observatory, that the mean value of this light is of the order of five times that of starlight. The total light falling on the earth on a bright moonless night may therefore be estimated to average in the neighborhood of 0.000,4 foot-candle.

On cloudy nights the light falling on the earth is of course considerably reduced in intensity. In order to calculate the amount of this reduction in light intensity I have measured the amount of reduction in photometer readings caused by the presence of clouds in daytime. For this purpose I used a Weston photoelectric exposure meter. A sheet of white typewriter paper was held in the hand and the maximum reading of the light from the sky reflected from this paper was read on the exposure meter, which was held at a distance of about one foot. Whereas the maximum reading on a sunshiny day in September was 800 foot-candles, that on a cloudy day varied from 80 to 50 foot-candles. This is a reduction to from 1/10th to 1/16th caused by the clouds. On a bright sunshiny day in March the maximum reading of the light reflected from the white paper was 650 foot-candles, while on cloudy days in the same month the readings varied from 50 down to as low as 20 foot-candles. These are reductions to 1/13th down to as much as 1/32nd.

If we assume a reduction in light intensity on a cloudy night of only one-tenth, the intensity falling on the earth under a full moon but with the sky covered by clouds would be 0.002 foot-candle, under a quarter-moon it would be 0.000,2 foot-candle, and on a cloudy, moonless night it would average in the neighborhood of 0.000,04 foot-candle. Under heavy clouds giving a reduction of the light to 1/32nd, the light intensity falling on the earth would be approximately 0.006 foot-candle at full moon but only 0.000,012 foot-candle on a moonless night.

In the habitats in which terrestrial animals live, the light that comes to the earth from the heavenly bodies is usually reduced through the shade cast by various objects, particularly by the vegetation. Under trees that have heavy foliage the reduction in light intensity is considerable. In order to measure the amount of reduction in light intensity caused by the presence of trees I have measured in summer the amount of light reflected from a white paper sheet when held under trees of various kinds compared to that reflected from the same sheet held in open sunlight. A Weston exposure meter was again employed as the measuring instrument.

In moderately dense oak-hickory woods on the George Reserve twenty-five miles northwest of Ann Arbor, Michigan, in September with the trees in full leaf the maximum light reflected from the white paper varied from 16 to 5 foot-candles. At the same time the white paper held in the open sun reflected 800 foot-candles of light. The reduction in light intensity under the shade of the trees varied therefore from 1/50th to 1/160th. Under a dense clump of shrubs a reading of only 4 foot-candles was obtained, which is a reduction of the light to 1/200th of its value in the open.

Under the shade of deciduous trees in summer, therefore, the light of the full moon would often be reduced to 0.000,2 foot-candle, if we assume a reduction to one per cent. due to the shade. The illumination would be still less than this amount if the shade reduced the light to less than one per cent. On a clear moonless night in summer the light falling on the ground in a deciduous forest would, according to the assumption of a reduction to one per cent., frequently be reduced to 0.000,004 foot-candle or less. On a cloudy, moonless night in summer the light reaching the forest floor must often fall to 0.000,000,4 foot-candle, if we assume a reduction to ten per cent. due to the clouds and to one per cent. of the remainder by the shade cast by the trees. Even less light might be available under a combination of heavy shade and very heavy clouds.

The density of shade in the places where these readings were obtained is by no means the maximum in the oak-hickory habitat. The method used is, however, not suitable for measuring the light intensity in small spaces, such as under the shade of logs or under small clumps of herbs. Nevertheless it is known that rodents and the other small animals that are the prey of owls often hide and travel in these spots of dense shade.

There are of course open spots in most types of forest. In such situations full sunlight may reach the ground at certain times of the day. Readings of the light reflected from the white paper in such sunny spots approached the readings secured outside the forest. Within the forest there is therefore practically complete gradation at different places and at different times from practically full sunlight to dense shade.

Other types of forest are known to produce still more dense shade than the oak-hickory. Grass, herbs, and brush also cast a considerable shade on the ground, though I have made no attempt to measure the amount of light intercepted by such types of vegetation. The light intensity falling on the ground in grass or brush-covered habitats must be intermediate between that in forests and that in completely open habitats.

The shade produced by deciduous trees in summer would seem from the above figures often to reduce the light intensity falling on the ground under the forest to one per cent. or less of that falling on bare ground. When the leaves are off the trees in winter the shade cast by the branches will of course only slightly reduce the light intensity. Coniferous trees undoubtedly cast more heavy shade than do deciduous trees, and because they do not drop their leaves in winter they are effective in reducing the light intensity at all seasons of the year.

The calculations made above are believed to give a rough indication of the amount of light available at night in certain types of habitat. It is not pretended that the figures of possible light intensities arrived at are in any way exact. Certainly there must be much variation from place to place and from time to time in the amount of light that reaches the ground in any given habitat. Also, it is not certain that the reduction in illumination produced by the shade of trees and by the presence of clouds, respectively, can properly be multiplied together as I have done. Furthermore, the light energy measured by the photoelectric cell is perhaps not derived from exactly the same wave lengths as those most used for vision by nocturnal birds and other animals. Nevertheless, it is certain that in some habitats in nature only extremely low intensities of light must at times be available for the use of nocturnal animals.

DISCUSSION

The question may fairly be asked whether in these experiments the owls actually secured the dead mice by the sense of sight or if some other sense or senses were employed.

It seems unlikely that hearing could have been employed in guiding the owls to their prey, because the mice were dead.

There is the possibility that supersonic sounds given off by the owl may be reflected back from various objects to the ears of the owl and thus enable the animal to learn of the presence of such objects. Such a special sense is suggested by the uncanny ability of most of the owls to fly back to their perch in complete darkness. The only owl that did not show this ability was the burrowing owl and this species very seldom left the ground. The perch in the reaction room was constructed in such a way that it could be approached only by flight. At first, if the light was extinguished when the barred owl or long-eared owl were on the floor of the room, they behaved awkwardly and in the darkness they flew against the sides of the room. Later, however, both of these owls and also the barn owl learned to fly regularly in complete darkness to the floor of the room and back to their perch. This ability, however, may be due to a good sense of position rather than to the perception of supersonic sounds. The fact that all the owls exhibited a greatly decreased ability to secure dead prey in the lowest intensities of light and in darkness, proves that supersonic sounds are at least of no great importance to these owls for finding their prey in dim light.

No evidence is given by these studies that the sense of smell is used by these owls to find their prey. Smell alone is certainly inadequate to enable the owls to locate their prey precisely in very weak light. Otherwise the birds should have been more successful than they were in finding their prey in very dim light and in darkness. In very dim light and in darkness several of the owls at various times actually touched with a wing or stepped upon a mouse carcass without securing it. If these owls use the sense of smell at all in locating the general position of their prey its value to them for the purpose must be very slight.

Infra-red rays are presumed to be used by an English species of owl (*Strix aluco*) in finding its prey (Vanderplank, 1934). The North American owls used in the experiments here reported, however, evidently were not able to locate their prey through the perception of infra-red light. Although the mice were freshly killed at the beginning of each experiment and therefore were still warm at that time, the heat rays that they gave off did not enable the owls to secure the carcasses regularly under conditions of very dim light. On one occasion two of the mice placed in the room were by mistake only stunned and were found still alive two and one-half hours later. Yet during this time the barred owl, which was free in the completely darkened room, failed to secure either of them. It seems certain then that infra-red rays are not used by these owls in finding either living or dead prey. These conclusions are in accord with those of Hecht and Pirenne (1940).

It seems necessary to conclude that under the conditions of these experiments the owls must have secured their dead prey largely or entirely by sight, except for the carcasses found by physical contact. Accordingly, the experiments may be assumed to give a rough measure of the ability of the owls to see their prey under various intensities of illumination.

An increase with practice in the ability of the owls to see in very weak light seems suggested in several of the experiments. In several instances the owls made better records in extremely dim lights late in a series of experiments than they had made in somewhat brighter light in the early part of the same series. These improvements, however, may only indicate an increase in the familiarity of the owl with the procedure of the experiment, rather than an actual increase by him in ability to see in weak light. The number of records are in every case few and therefore do not conclusively demonstrate such an increase in the visual ability of the owl with practice.

The experiments here described demonstrate that the barred owl, long-eared owl, and barn owl have somewhat the same lower limit of vision in dim light. All these species seem able to see their prey without difficulty at a distance of six feet or more when the illumination reaching the ground is about 0.000,000,73 foot-candle or more. Below this level of illumination all three species probably have difficulty seeing prey at a distance. Thus, the barred owl was able to pounce directly on her prey when the light intensity falling on the floor of the reaction room was calculated to be 0.000,000,73 foot-candle. But when the light intensity was reduced to 0.000,000,53 foot-candle the bird seemed able to detect the prey only when she was within a foot or so of it. The long-eared owl was able to pounce directly on the dead mouse when the incident light measured 0.000,001,05 foot-candle, but at an intensity of 0.000,000,25 foot-candle he found the carcasses only by searching. The barn owl was undoubtedly able to see objects at a distance of six feet or more at a light intensity of 0.000,002 foot-candle, but he had some difficulty finding the carcasses when the illumination was reduced to 0.000,000,53 foot-candle. The data, therefore, do not demonstrate any very important difference between the ability of these three species of owls to detect prey at a distance of six feet or more.

The ability of the owls to see prey at very close distances in very dim light however, may differ slightly between these three species. It is not certain that the barn owl is able to perceive near-by objects at all at a light intensity of less than 0.000,000,53 foot-candle. The long-eared owl is probably able to perceive near-by objects at least dimly under illuminations as low as 0.000,000,25 foot-candle. The barred owl, when given an abundance of time, may be able to detect near-by dark-colored objects on pale-colored soil under an illumination of as little as 0.000,000,15 foot-candle.

If these figures can be relied upon, the barred owl has a slightly better vision for dim light than the long-eared owl, and the long-eared owl has slightly better night vision than the barn owl. Such a conclusion would agree with the general observation that the barred owl is an inhabitant of somewhat denser woods as a rule than either the long-eared or barn owls. However, it should be emphasized that the experiments in the dimmer illuminations were much more extensive on the barred owl than on either of the other species. Also it should be repeated that the 20-minute test period mostly used for the barn owl is not fully comparable with the all-night period used with the other owls.

The illumination at which these owls are able to see their prey seems very dim when judged by the human eye. A light intensity of 0.000,000,73 foot-candle falling on the floor of the reaction room would be the amount of illumination produced by a standard candle at a distance of 1,170 feet. Yet these three species of owls were evidently able to see their prey very well at a distance of six feet or more in this amount of light. An illumination of 0.000,000,53 foot-candle would be produced by a standard candle at a distance of 1,374 feet. In such weak illumination these owls seemed still able to see objects that were about one foot away. A standard candle 2,000 feet away gives an illumination of 0.000,000,25 foot-candle, and one 2,582 feet distant produces an illumination of 0.000,000,15 foot-candle. The evidence from these experiments indicates that the barred owl was able sometimes to detect and to secure dead mice in these amounts of light. But in the amount of illumination calculated to be given by a standard candle at 3,536 feet (0.000,000,08 foot-candle) no evidence was found that even the barred owl was able to see anything.

It is not easy to compare the vision of an owl with that of a man. A few observations that attempt such a comparison are, however, here presented. My own eyes, somewhat dark-adapted by a period of five minutes or more in darkness, were able to detect a dead white mouse placed on dark-colored soil on the floor of the reaction room under an illumination calculated to be 0.000,003,5 foot-candle. At this illumination, however, the outlines of the mouse could not be seen and there was only an indistinct blur of whiteness toward the side of my visual field, even when my eyes were within a few inches of the carcass. I probably would not have discovered the mouse if I had not known exactly where it was. At a slightly lower level of illumination only a faint glow of light could be detected at the edge of my visual field. When the light intensity was increased to a calculated 0.000,015 foot-candle I was able to see the mouse as a white blob. When the illumination was further increased to 0.000,026 foot-candle I was able to see the outlines of the mouse, though still not clearly. Several other persons tested in the reaction room under similar conditions had a lower limit of vision approximately equal to my own. We may therefore conclude that barred, long-eared, and barn owls are able to see objects under an illumination of between one-hundredth and one-tenth of the light intensity that man requires for vision.

This conclusion agrees reasonably well with the calculations of Hecht and Pirenne (1940). From a study of the brightness that produced the minimal effect on the iris of the long-eared owl these authors calculated that this species could see in light 1.5/4.0th that required by man. They, however, agreed that this figure might be off by a factor of 10.

The burrowing owl differs considerably from the other three species of owls tested in its ability to see in dim light. Under the conditions of our experiments the burrowing owls were unable to find dead mice regularly in a light intensity of less than 0.000,026 foot-candle. The burrowing owl, therefore, seems to have somewhat the same ability to see in weak light as does man. It is not nearly so well adapted for vision in dim light as are the other three species of owls under consideration.

This poor adaptation of the burrowing owl for vision in weak light is correlated with its habits. The burrowing owl is much more diurnal in habit than are the other kinds of owls tested. It is not usually active during the brightest part of the day, but it may often be seen moving about in the early morning and in the evening.

Attention has already been called to the fact that the owls seemed able to secure prey somewhat more readily on the pale-colored silica sand than on the darker colored soil. This effect is presumably due to the light reflected from the nearly white silica sand giving a slight increase in overall illumination in that compartment as compared to the compartment with the dark-colored soil.

The computation of the minimum illumination at which the owls were able to find their dead prey has in each case been based on the most favorable conditions. Usually the owls were more successful in securing gray mice on the silica sand than they were in securing ivory or albino or gray mice on the gray gulley soil. The minimum illumination at which the owls would be able to see concealingly colored prey or to find prey of any color on dark-colored soil would undoubtedly be greater than the amounts in foot-candles stated above.

There is a suggestion in some of the experiments that mice having shades of color contrasting with the soil on which they were placed were taken somewhat more readily than those that more closely matched the soil color. These experiments, however, cannot be considered to be a measure of the effectiveness of concealing coloration in protecting these mice from their predators. Such a measure would only be valid if the mice were alive, which these were not. I have, therefore, not made any effort to calculate the proportions of the colors of mice taken on the soils of matching and of contrasting color. Other experiments, designed to measure the effectiveness of the protective coloration of live Peromyscus against owls, are now in progress and will be reported upon later.

The experiments here reported give no information about individual, sexual, or seasonal variation in the ability of owls to see in weak light. Only two burrowing owls were tested and no attempt was made to separate the records made by each individual. Of the other three kinds of owls studied only a single individual of each species was observed. It is obvious, therefore, that the measurements of light discrimination obtained may not well represent the abilities of other individuals of these species.

Furthermore, additional observations of each of the individual owls studied would have been desirable, especially at the lower light intensities near the critical limit of the owl's vision. However, these experiments are very time-consuming, as will be recognized when it is considered that an average of only about one record can be secured per day. The methods employed are crude and the light intensities could not be controlled fully accurately nor could they be precisely measured. Nevertheless, it is believed that the data presented offer convincing evidence that certain kinds of owls can see their prey in light of extremely low intensity.

The barred, long-eared, and barn owls are in general inhabitants of forest, though some of them at times undoubtedly forage in adjacent open fields. In an earlier section of this report it has been calculated that the amount of light falling on the floor of a deciduous forest in summer may amount to 0.000,004 foot-candle during a clear moonless night in summer. All these owls would be able to see their prey readily in light of that intensity. On cloudy nights, however, the illumination on the floor of a deciduous forest must often fall below 0.000,000,4 foot-candle, and in this light intensity the owls would not be able to see their prey at a distance of more than a few feet at most.

The burrowing owl lives in open fields and makes its home in burrows in the ground. Under heavy clouds on a moonless night the light falling on the earth must nevertheless often be reduced to 0.000,012 foot-candle or less. This is below the limit of illumination at which the burrowing owl can readily see its prey. Furthermore, the small animals on which the burrowing owl feeds often keep under the shade of grass, herbs, and small shrubs, all of which further reduce the amount of light reaching the ground.

In nature, therefore, all the species of owls must frequently encounter intensities of illumination that are below the minimum at which they are able to see non-moving prey.

Moving animals are more readily detected by sight than non-moving ones, as any field naturalist can attest. It is therefore likely that an owl would be able to preceive living, actively moving prey at a somewhat lower intensity of illumination than the dead prey employed in these laboratory experiments. However, many kinds of animals assume a stationary, rigid attitude ("freeze") when startled or frightened. It may be assumed therefore that an owl, in order to be sure of securing his prey, must be able to see non-moving prey individuals as well as ones in motion. Furthermore, it seems certain that the lower intensities of illumination reached in nature must often extend well below any possible level of owl vision, even for moving objects.

SUMMARY

All four species of owls studied in the laboratory found their dead prey mostly by sight and there was no evidence of their employment for this purpose of infra-red rays or of any senses other than sight and physical contact.

The barred, long-eared, and barn owls are able, under the most favorable conditions, to see and to approach dead prey directly from a distance of six feet or more under an illumination calculated to be as low as 0.000,000,73 foot-candle. When the illumination is reduced to 0.000,000,53 foot-candle, however, all these owls seem to have some difficulty in seeing prey that is more than a foot or so away. There is some evidence that sight may be of some value to the barred owl in finding dark-colored dead prey on nearly white soil at an illumination as dim as 0.000,000,15 foot-candle.

The burrowing owl was unable to find dead prey regularly under illuminations dimmer than about 0.000,026 foot-candle. This species, therefore, has much less ability to see in very dim light than the other three species of owls studied. The burrowing owl, however, is more diurnal in habit than these other owls and also it lives in more open situations.

From measurements of the incident light in nature it is calculated that in the natural habitats of all these owls the intensity of illumination must often fall below the minimum at which the birds can see their prey.

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THE STERILIZING EFFECT OF HIGH TEMPERATURE ON THE MALE GERM-PLASM OF THE YUCCA NIGHT LIZARD, *XANTUSIA VIGILIS*[1, 2]

PROFESSOR RAYMOND B. COWLES AND G. L. BURLESON

DURING the past decade, evidence has been accumulating on a curious phenomenon of thermal differentiation, an apparent conflict between temperatures which may be tolerated or even required by body cells, versus those which may be deleterious to the germ cells.

Most of the earlier work has dealt with the condition as it is encountered in the mammals, but later studies, some of them possibly still inconclusive, are strongly indicative of the possibility that in a majority of the terrestrial animals there may be a similar thermal hiatus between groups of cells.

In every instance so far reported, the body cells of terrestrial animals endure or even require temperatures that are markedly higher than those which can be tolerated by their germ cells. This would seem to be a highly significant phenomenon in view of the fact that there is an almost universal rise in temperature requirements which keeps pace with phylogenetic ascent, and that the either conservative or heat-sensitive germ cells must have kept pace also.

It is well known that in the scrotal mammals, even normal body temperatures are high enough to cause sterility; therefore, by analogy, if parallel conditions existed in any of the cold-blooded animals, optimum temperatures would result in their automatic sterilization. Even less extreme effects resulting from heat sensitivity of the germ cells have dramatic possibilities in connection with the influ-

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[1] These investigations were made possible through grants in aid of research provided by the Regents of the University of California.

[2] I wish to express my great appreciation to the junior author who is responsible for all the histological and photographic work involved in the preparation of this paper.—R.B.C.

ence that this trait may have had on evolution, and these possibilities may be enhanced by the comparatively recent discovery (Plough, 1942) that abnormally high but substerilizing temperatures in at least one animal, *Drosophila*, may increase the rate at which mutations appear.

The evolutionary possibilities inherent in this nearly universal duality of thermal requirements have been tentatively presented by Cowles (1944), but while engaged in studies of the available information and the formulation of concepts relative to the effects of thermal problems, it became clear that fuller understanding and added security of conclusions could be obtained only through knowledge of the conditions found in the reptiles. This group of organisms is particularly significant because of their position midway between the aquatic progenitors of land forms, and their ultimate product, the thermally stable warm-blooded birds and mammals.

THE YUCCA NIGHT LIZARD

Xantusia vigilis was chosen as a representative of the Reptilia, in part because of the possibility that only nocturnal or tropical reptiles may have retained the thermal condition most like that of their progenitors, but chiefly because the normal spermatogenic cycle was already available through the work of Miller (1942) and secondarily because of its abundance and availability throughout the year.

As the vernacular name implies, this is a nocturnal lizard with a preferred habitat consisting of groves of Joshua trees (*Yucca brevifolia*) or in some places, stands of the Mojave yucca (*Yucca mojavensis*). More rarely the lizards may be found under the dead leaf rosettes of the Spanish bayonet (*Yucca whipplei*), the Agave (*Agave deserti*) and Nolina (*Nolina parryi*) as well as under flat rocks, especially exfoliating granite. Sometimes it is also found under boards, papers and other trash which has become matted to the ground. Geographically, its range is confined chiefly to the Mojave desert, but to the southward in California there is a limited extension of its range along the higher flanks of desert mountains.

The adult male lizards average approximately 38 mm, snout to vent, or 70 mm in total length, while the somewhat larger females are approximately 42 mm, snout to vent, or about 85 mm in overall length. Differences in size are neither sufficiently obvious nor constant to afford a ready means for recognition of the sexes. During the spring months, when testicular activity is greatest the femoral pores of the males are enlarged so that they give the legs a somewhat angular cross-sectional outline; nevertheless, even after great care has been exercised in segregating the sexes, a few mistakes will almost inevitably occur.

Adult lizards are always obtainable and there is no evidence of true hibernation, although the congregation of 4 to 8 or more lizards in especially suitable wood-rats' nests[3] suggests that a few individuals may pass into relatively continuous torpor. Most of the lizards seem to remain scattered singly under decaying logs of the Joshua trees, and during spells of warm weather, these animals move out from under the logs and climb up between the trunk and loose bark and foliage. In this manner, these dark-loving lizards can find suitable temperatures during the warmest part of the day, without being exposed to light, and they are thus capable of normal activity even during the relatively cold winters.

These lizards seem to be particularly abundant in the Antelope Valley, a westward extension of the Mojave desert, and in this area winter temperatures often fall to - 10° C., sometimes even lower; but these cold intervals are usually short, and the extremes of low temperatures are usually confined to the nights. Even during the coldest weather, the temperature under the logs occupied by the lizards rarely falls lower than 7 to 8° C., and during the daytime, temperatures just beneath the bark of the decaying logs may rise to 20° C., sometimes more.

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[3] I am indebted to Mr. Charles Lowe for this information.

Throughout the winter and even as late as April, there are occasional snowstorms or very cold rains that temporarily immobilize the lizards even during daytime. As late as May, night temperatures may fall so low that during the early morning hours the lizards are sluggish.

A body temperature of 0° C. kills these lizards in a few hours' time, but it is probable that only stragglers, or more rarely those living in marginally suitable shelters, suffer from the fatal effects of cold weather.

Laboratory studies of auto-selected temperatures seem to indicate that there is no sharply marked thermal preference, or optimum, within the broad range extending from 25° to 30° C. Temperatures above and below these points seem to be only slightly less satisfactory since the lizards have been observed, apparently at rest, in parts of the experimental cage where air temperatures were as low as 20° C. and as high as 37° C. In one instance three of 20 specimens in a cage were found hiding behind an ice-filled carton where the temperature was 12° C.

An air temperature of 45° C. causes death in 35 minutes, and at the onset of death struggles, thermocouple readings indicated that the body temperature had risen to only 38° C. An environmental temperature of 40° C. causes 50 per cent. mortality within three hours, a notable fact since temperatures from 40° to 45° C. are a daily occurrence in their near-environment from June to late September.

Like all others on which information is available, these lizards are exceedingly susceptible to temperatures only slightly above those which they will tolerate voluntarily, and after they have been removed from their sheltered hiding places it is necessary to protect them from the normal summer heat. Even a relatively short exposure to 39° C. is so rapidly fatal that great care must be exercised to keep them cool, and transportation from the desert to the cooler coastal areas often constitutes a major collecting hazard. If the animals are supplied with drinking water, approximately 50 per cent. will survive 38° C. for a week, while without water not more than 30 per cent. appear to be capable of enduring the same temperature for only 30 hours. This difference may be due to dehydration resulting from evaporation of moisture used in respiratory cooling activity, or it may be due to either the failure of respiration or of the cooling mechanism when unable to replenish lost moisture. A body temperature of 39° C. can safely be accepted as the lethal temperature.

A week of exposure to environmental temperatures ranging between 37° C. and 37.5° C. killed only apparent weaklings, those with abnormally slender tails or those with a generally emaciated appearance, and we have, repeatedly maintained numbers of the animals for a week or more at 36° to 36.5° C. with no losses.

The experimental animals were supplied with food and water at all times except in the tests reported above. The most successful item of diet was the dry wood termite. *Kalotermes minor.*[4] These were always taken with avidity, as were other, but unidentified species that were only infrequently available. Small larvae of *Tenebrio molitor* were acceptable for short periods, but the lizards soon refused the meal worms. All the animals, including those used in the heat treatment required by the experiments, throve and gained weight. In almost all cases the diameter of the tail increased noticeably, a sure indication of well-being, and on dissection, practically all had large lobules of adipose tissue in the abdominal cavity. Many of the lizards were retained from early April until October 15, and almost all were still in excellent conditions. It is notable as evidence of normal conditions that captive females gave birth to their offspring, both twins and single young, at the same time that freshly captured animals were giving birth to theirs (Cowles, 1944). Both the adults and young showed no effects of the long period of captivity, and they were indistinguishable from the wild stock.

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[4] I am indebted to Dr. Gordon H. Ball for the identification of these termites.

NORMAL SPERMATOGENESIS

According to Miller (*supra cit.*) and our own findings, male lizards taken in January show signs of sexual development. This is indicated by the appearance of numbers of mitotic figures in the 3 to 4 rows of peripherally located spermatogonia, and in the 5 to 6 layers of centrally placed spermatocytes. Development throughout February and early March is normally confined to multiplication of these cells, but by late March spermatids are forming. The spermatocytes and spermatids continue to multiply so that by late April only one or two rows of spermatogonia remain, but it is not until mid-May that the spermatids start metamorphosis and transform into the first spermatozoa. By late May the transformation is virtually completed and practically all the cells have reached either the spermatid or spermatozoan stage. Testes remain in this condition until about mid-June, when the initial stage of collapse appears, and by the end of June they are reduced in size and no spermatogenic activity is visible. The testes remain in this inactive state throughout the summer.

Fecundation of the females appears to take place between mid-May and mid-June, and gestation requires approximately 3½ to 4 months (Cowles, *supra cit.*); thus the young are not born until early autumn.

Following the June collapse, the testes remain in a quiescent condition until the following August, when they again show signs of activity as indicated by a notable increase in size which is accompanied by the appearance of numerous mitotic figures, and production of primary and secondary spermatocytes, and what appear to be scattered spermatids. It is possible that a very few spermatozoa might occasionally be produced, nevertheless the testes that we have observed do not appear to reach such a full development as occurs in spring.

Progress toward the development of a fully functional testis may be prevented by the inevitable onset of cold weather, but in any event no additional growth occurs until the ensuing spring.

The autumnal resurgence in activity appears to be a direct result of moderating summer temperatures, and lengthening of cooling nights and the onset of cold weather, but it is recognized that this may be due to coincidental association of weather and some cyclic phenomenon.

That regeneration is not necessarily a fortuitous juxtaposition of unassociated events is suggested by evidence from other sources such as the studies of Rahn (1940) on the viability of sperms in the uterus of the garter snake *Thamnophis,* and Trapido (1940) on sperm viability in *Storeria.* The observations of Blanchard and Blanchard (1942) on *Thamnophis s. sirtalis* give conclusive evidence for an autumnal resurgence in sexual development in the males, which seems to be postulated on the occurrence of an interval of cold weather followed by Indian summer. Fertile copulations occur, as are demonstrated by birth of normal broods the following spring. There is a strong implication that similar conditions may be found in the desert rattlesnakes, *Crotalus cerastes* and *Crotalus atrox,* as reported by Lowe (1942). These snakes, like*Thamnophis* and *Storeria,* normally breed in the spring of the year.

EXPERIMENTAL PROCEDURE AND RESULTS

Testing for the possible existence of thermal divergence was a simple process, since the animals were merely placed in a thermostatically controlled heating chamber and were then allowed to move about within the confines of a smaller centrally placed screened box where previously conducted tests and successive check observations showed fluctuations of only ± 0.5° C. Because of the nocturnal character of the lizards, a feeble light of 5 cp was employed whenever observations were necessary. Humidity was not controlled, but the animals were provided with a constant supply of water and displayed no signs of discomfort.

No attempts were made to record body temperatures during these experiments, since it was deemed advisable to disturb the lizards as little as possible. Furthermore, previous studies have given satisfactory evidence that these animals are capable of some body cooling, which may amount to as much as 1.5° C. under ideal conditions of high temperature and low humidity. Under no circumstances have body temperatures risen above those of the environment, therefore there was ample assurance that body temperatures would remain at or slightly below those of the testing cage.

Throughout the entire period of investigation extending from January to early May, and from late May to October, 1943, all controls with the exception of sick or feeble animals (about one in fifty) maintained the normal testicular cycle as determined by microscopic sections obtained from wild animals collected at bi-weekly intervals. Control animals were kept in the laboratory at room temperatures ranging between 25° and 27° C. from January until early May when the laboratory temperature rose to 30° - 32° C. for about ten days.

The experiments were designed primarily in order to determine whether or not high temperatures might prevent the production of viable sperm or cause either lasting or transient damage to the spermatogenic organs and mechanism.

In order to exclude the possibility that heat might have less drastic effects at different stages of development it was necessary to discover whether or not heat damage could be produced at any stage of testicular development, particularly during the last stages in the process which occur just prior to the onset of high daytime temperatures and warm night conditions when the temperature of the soil is also rising rapidly.

Additional objectives of these experiments were to discover whether or not heat damage might not be sufficiently transient as to allow rehabilitation and reproduction during the same season, and whether or not collapse could be prevented or recuperation might not be accelerated by exposure of the animals to low temperatures comparable to those encountered by the animals in nature during winter.

This possibility of recuperation was in part suggested by the work of Wells (1935 a and b) on the thirteen-lined ground squirrel, *Citellus tridecemliniatus,* a seasonal breeder in which normal summer collapse of the testes can be prevented by placing the animal in a cold environment.

In an experiment devised to test these possibilities ten specimens of *Xantusia vigilis* which had been heat-treated and ten which were going through their normal cycle were tested to determine whether low temperatures would prevent immediate collapse of testes in the former and ultimate collapse in the latter. The experiment was a failure; probably due to inadequate facilities for maintenance under the desired conditions which made it necessary to use 12° - 13° C. in place of the needed 15° - 20° C. with interrupted exposure to as much as 27° C. Collapse of the testes was postponed six weeks beyond expectancy, but this may have been due to a retardation of normal progress. It is notable that gravid females maintained during this period under the same conditions as the males all yielded only abnormal embryos.

The effects of treatment at 38° C. for one week result in progressive atrophy and it is doubtful if recovery can take place following such drastic effects as those shown in the sequence of figures in Plate I. The testes show notable signs of extreme damage at the end of the first week of exposure. Most of the spermatogonia and primary spermatocytes characterizing the normal March testis show either agglomeration or disintegration of the nuclear material and macrophage activity is notable. Following the first stages of progressive atrophy the tubules show cloudy degeneration and finally almost complete atrophy sets in.

The effects of 36° C. as applied to the late March testis

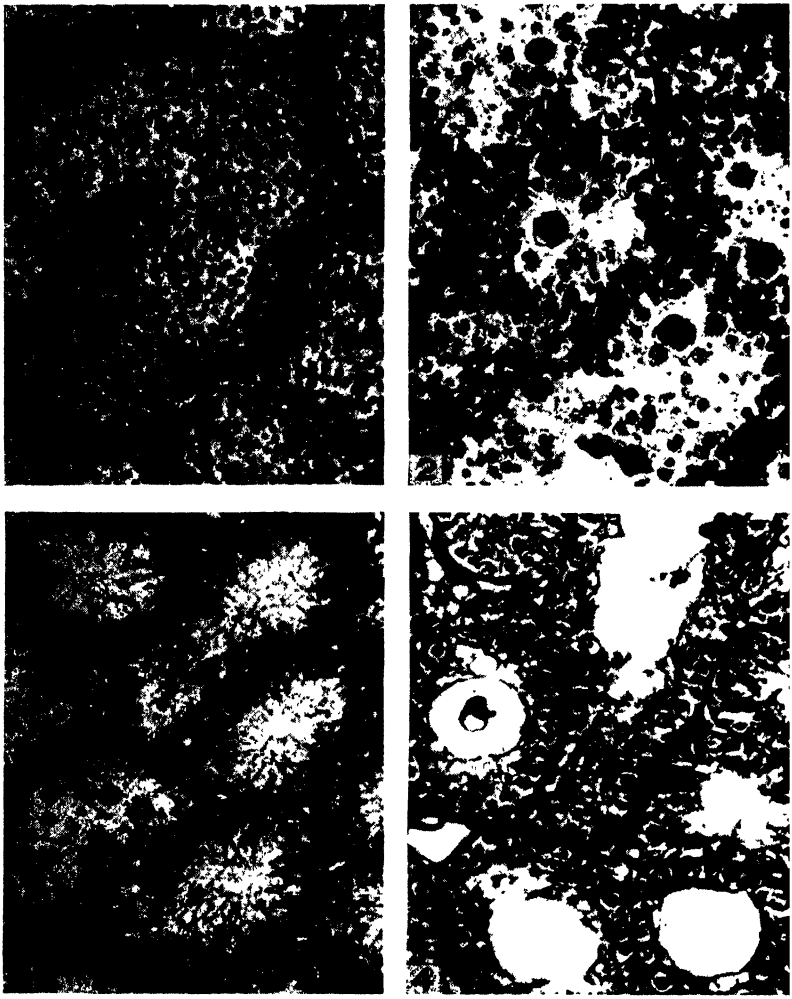

PLATE I. Effects of heat (environmental temperature of 38 ± 0.5 for one week) on early March testis.
FIG. 1. Normal testis. March 8, 1943. × 225.
FIG. 2. Testis after treatment for one week. × 225.
FIG. 3. Testis two weeks after heat treatment. × 225.
FIG. 4. Testis three weeks after heat treatment. Atrophy. × 225.

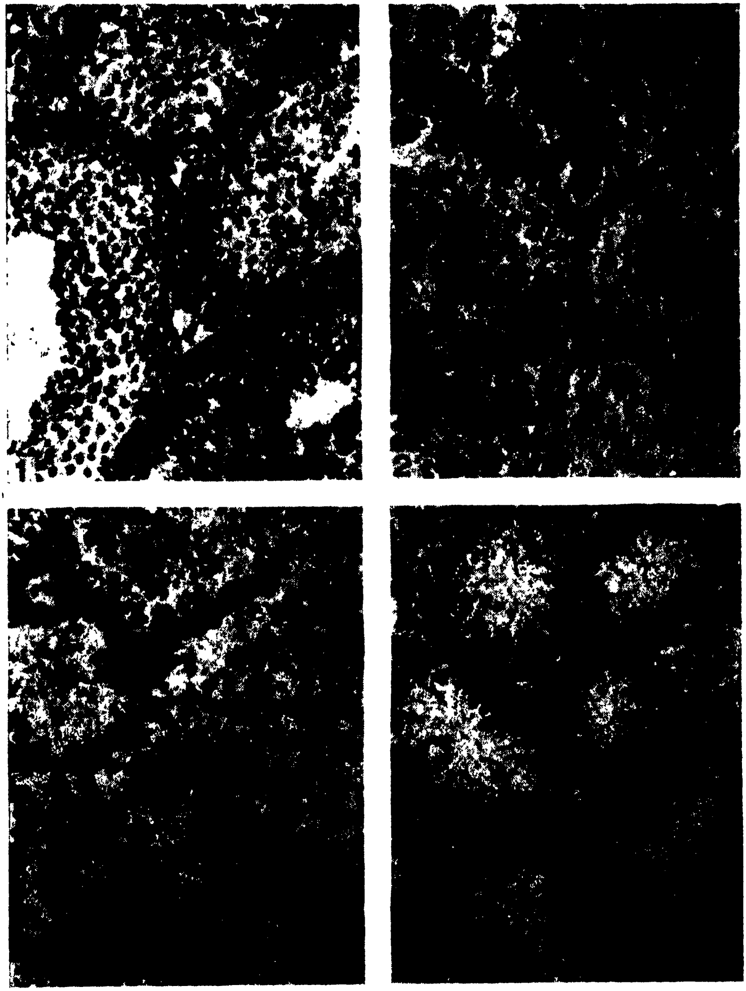

PLATE II. Effects of heat on late March testis, 36 ± 0.5 C. for one week.
FIG. 1. Normal testis. × 225.
FIG. 2. Testis at end of one week. × 225.
FIG. 3. Testis at end of two weeks. × 225.
FIG. 4. Testis at end of three weeks. Recovery, cf. Plate V, fig. 2. × 225.

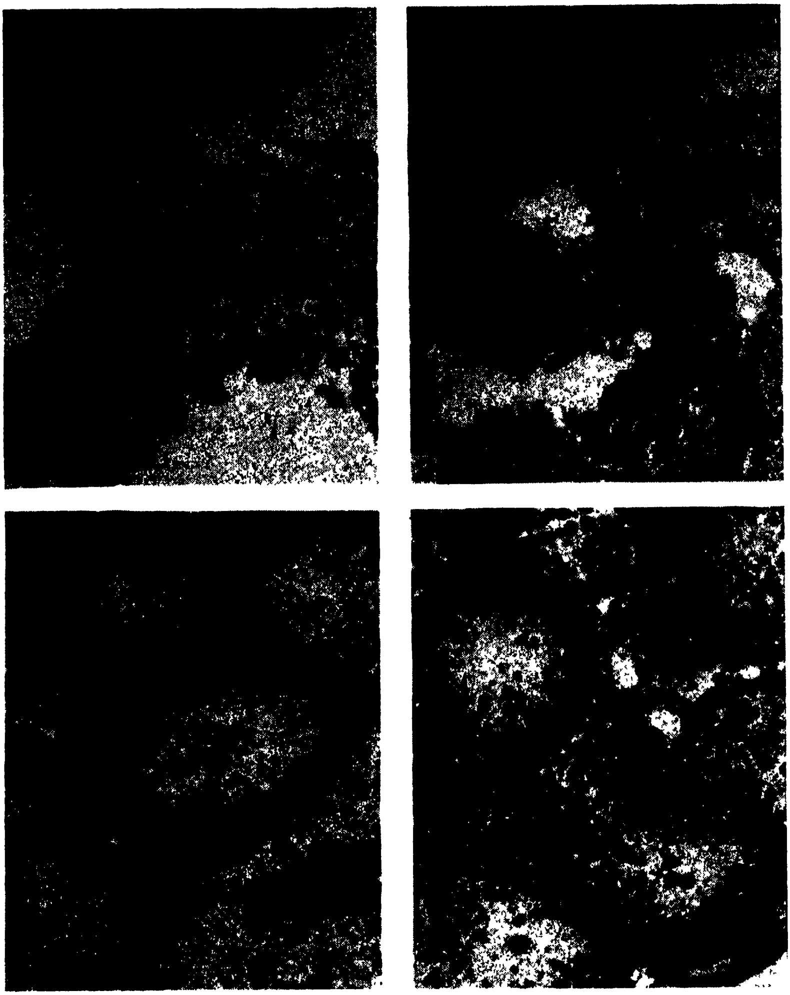

Plate III. Effects of heat on late April testis, 36 ± 0.5 C. for one week.
Fig. 1. Normal testis. × 225.
Fig. 2. Testis at end of one week. × 225.
Fig. 3. Testis at end of two weeks. × 225.
Fig. 4. Testis at end of three weeks. Recovery, cf. Plate V, fig. 2. × 225.

result in extensive destruction of the spermatogonia and disintegration of all the primary spermatocytes. Macrophage activity is conspicuous and, though disintegration is extensive, recovery starts during the third week following heat treatment and the tubules assume the normal summer condition encountered by late June or mid-July. These stages are shown in Plate II, Figs. 1–4.

The effects of 36° C. on the late April testis, Plate III, Figs, 1–4, are similar to those of earlier date, but it is notable that the effects of heat are extended to include destruction of the rapidly multiplying spermatids, and secondary spermatocytes, as well as the earlier stages involved in the less advanced testis. Recovery appears to be somewhat more rapid (*cf.* Plate II, Fig. 3), but does not advance beyond the summer state of inactivity, shown in Plate V, Fig. 2.

Even the most advanced stage of testicular development, found in mid-June, suffers from the effects of a week's exposure to a temperature of 36° C. (Plate IV, Figs. 1 and 2, and 3 and 4); but in this case there is a notable inclusion of the transforming spermatids and mature spermatozoa, as well as all the earlier stages in the destructive effects of heat. The gross effects on size of the testes are most clearly observable in Plate IV, Fig. 1, the normal testis and Fig. 2, the shrunken, remains two weeks after treatment. Figs. 3 and 4 illustrate the histolytic effects of heat.

Although the testes of control animals kept at 25° – 27° C. were indistinguishable from those of freshly caught material, a continuous check was carried out in order to avoid any possible errors due to unexpected changes in the controls which might result from the effects of either laboratory temperatures or unusual light or feeding activities.

As long as the temperatures remained 25° – 27° C., no difficulties were encountered, but early in May a hot spell kept laboratory temperatures between 31° C. and 32° C. for almost two weeks. Under these conditions the testes

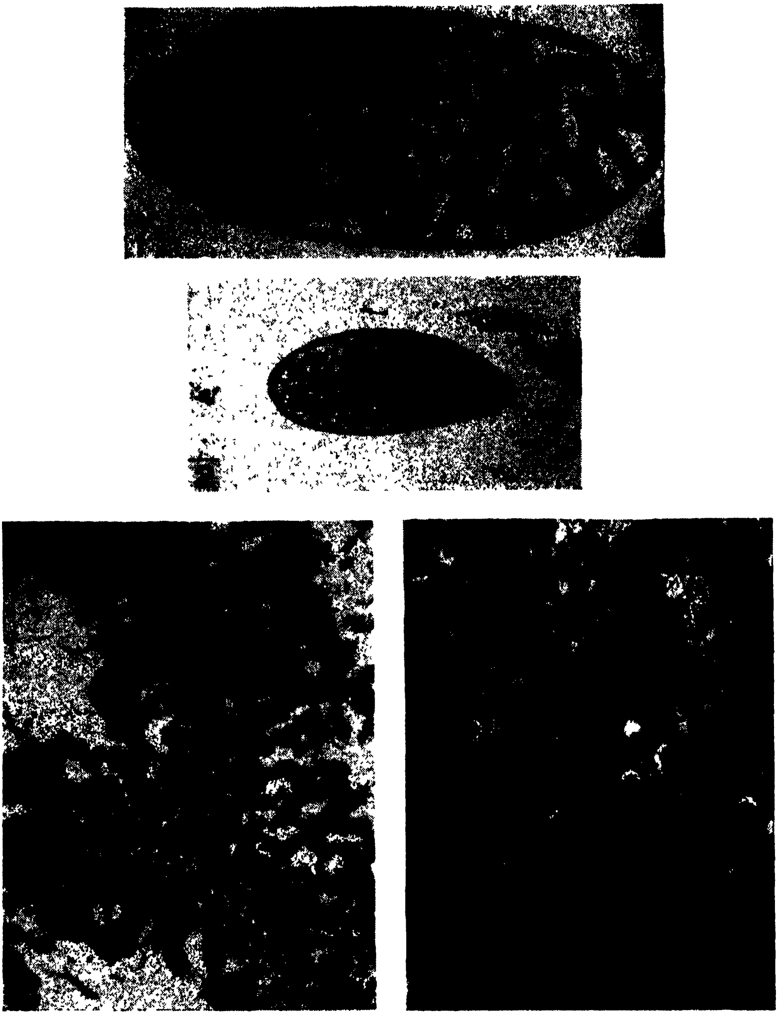

PLATE IV. Effects of heat on mid-June testis, 36 ± 0.5 C. for one week.

FIG. 1. Normal testis at period of maximum development. × 33.

FIG. 2. Heat treated testis showing diminution in size. × 33.

FIG. 3. Histological appearance of testis shown in fig. 1. × 225.

FIG. 4. Histological appearance of testis shown in fig. 2. × 225.

of control animals showed the first signs of abnormality (Plate V, Fig. 1, compared with its freshly caught counterpart, Plate IV, Fig. 3).

There is little reason to doubt that the comparatively moderate temperatures of 31° – 32° C. were responsible for the slight disintegration shown in this control animal, making studies at this level particularly interesting since the lizards are probably subjected to similar temperatures for at least part of the day during late June and early July, the time for normal collapse, and may even come to rest at these temperatures under experimental conditions.

One of the most interesting problems for future investigation is suggested by the possibility that these lizards may either voluntarily or of necessity submit to temperatures that would prevent breeding if encountered earlier in the season.

From May 17 to 24, five individuals were subjected to treatment at 36° C., and these were then kept at room temperatures until August 27, when they were killed and the testes compared with freshly caught material. The result of this experiment is shown in Plate V, Fig. 3, where the experimental testis has shown complete recovery and is no longer distinguishable from the testes of freshly obtained animals, Fig. 4. There can be little doubt but that normal reproductive activity would be possible the ensuing spring.

DISCUSSION

From the foregoing reports on the effects of high temperature on the reproductive cells of the male *Xantusia vigilis* it is apparent that even moderate and somatically harmless heat produces complete sterility, at least for the season in which it occurs. It is not known how permanent the effects of 38° C. may be, nor whether there may be other and more subtle damage at lower levels, but it appears probable that the appreciable and obvious damage resulting from a week of exposure to 36° C. is transi-

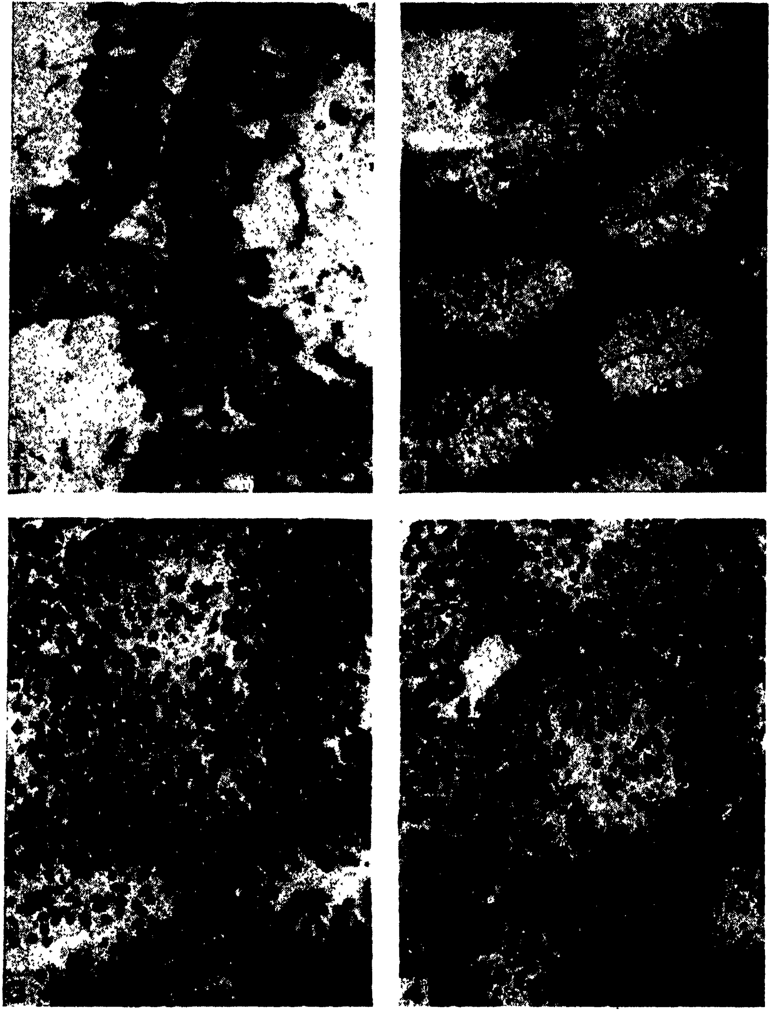

Plate V. Heat responses of testes of late spring, summer and fall.

Fig. 1. Testis of control killed May 18 after slight heat damage. x 225.

Fig. 2. Normal testis of mid-July for comparison with fig. 1. x 225.

Fig. 3. Recovery of testes treated at 36 May 17–24. August 27. x 225.

Fig. 4. Recovery of testis after normal collapse. August 27. x 225.

tory, and that recovery from the gross effects is completed by the time of normal autumnal testicular resurgence. Whether or not more subtle changes have taken place can only be determined by breeding experiments that may require a number of years for completion.

Because of the universal testicular collapse in nature, and the correlation of this phenomenon with the onset of high summer temperatures, and because of the similarity of normal and experimentally produced failure, it is natural to inquire as to whether or not the strictly seasonal nature of the gonadal cycle may not primarily be due to temperature effects. This view is supported by the autumnal, cool weather, resurgence in *Xantusia vigilis,* and is given additional indirect support by the reports in the previously cited papers of Blanchard and Blanchard, Rahn, Trapido, Lowe and Wells.

The nature of thermal susceptibility is still unknown, but it seems possible that the temperature vulnerability of the male germ cells may be inherent in either the active processes which accompany spermatogenesis, or it may be due to the existence of special properties possessed by these cells only, and not by their somatic vehicle. In this connection it seems particularly significant that some cells, apparently in the peripheral layer of the tubule, the quiescent areas, remain apparently unaffected so far as can be learned, therefore it would appear most probable that vulnerability is associated with rapid cell division. This may be due to a transient difference in the biophysical-biochemical composition of the active cells, or to the over-acceleration of an already superactive process. It is significant that rehabilitation of the tubules comes from the cells which were least active at the time of cellular heat damage.

It is extraordinarily interesting and probably significant that the temperature level affecting the germ cells of one class of organisms does not coincide with that affecting others, but that in each case the level of susceptibility appears to be correlated only with the normal somatic tolerance of the species. This situation suggests that in the process of evolution and progressive adoption of higher temperatures the male germ cells of each group have been required to make compensatory advances in temperature tolerance in order to keep pace with the advances of the somatic cells. That no animal yet studied appears to have coordinated the temperature limitations of the two groups of cells at present gives the appearance of a reluctant progression in one group and active advances in the other, resulting in an ultimate state of thermal compromise having many trenchant actual and theoretical possibilities.

Because of the possibilities inherent in this phenomenon, it would be desirable to learn whether or not the variations produced by mutations, and necessary for evolution by selective survival, affects the two classes of cells simultaneously, or whether they may be, in a sense, separable. Is there by any chance a separate field of mutations that may adjust the spermatogenic cells and processes to the changes adopted by their somatic vehicle, and have these changes failed to maintain thermal harmony? If this is the case, it would appear that the germ cells must await the remote chance of a purely random mutation leading toward closer thermal harmony, and without this accident the organism must remain the site of a potential conflict of thermal interests, thus existing, in a manner of speaking, in a state of civil war.

Whatever the mechanism may be, it seems to be an inescapable conclusion that the existing, and apparently nearly universal temperature divergence as found in the terrestrial animals must have been involved in the process of evolution on many occasions. The continued existence of many mammals seems to be predicated on the basis of a scrotal or other testicular cooling device, and the mutational steps leading to these developments can not be considered as a simple preordained straightforward progress to this specific solution. The conflicts and difficulties encountered by the reptiles and birds and their solution of them still constitutes an unknown field.

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LIFE TABLES FOR THE BLACK FLOUR BEETLE, *TRIBOLIUM MADENS* CHARP.[1]

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I

ALTHOUGH scattered observations on the life span of many vertebrates and invertebrates are legion, the accurately collected mortality data necessary for the construction of a life table are, apart from man, few indeed. For the insect group, life tables in complete or abridged form have been published for representatives of four orders: the Orthoptera (*Blatta orientalis*), the Lepidoptera (*Telea polyphemis* and *Acrobasis caryae*), the Diptera (*Drosophila melanogaster* and various of its mutants), and the Coleoptera (*Tribolium confusum*). Reference to the first three orders will be found in the papers of Pearl and Miner (1935 and 1936); to the last order in Pearl, Park and Miner (1941). The present paper reports a life table for another, and hitherto little studied, flour beetle, *Tribolium madens*. The details of life table construction, the significance of the life table for many phases of population biology and a review of the literature dealing with mortality in infra-human populations are not considered pertinent for this short paper. If interested, the reader can find further information in Bodenheimer (1938), Dublin and Lotka (1936) and Pearl (1940).

The flour beetles (*Tribolium confusum* and *Tribolium castaneum*) are proving to be appropriate organisms for experimental populations studies (see Ford, 1937, and Park, 1941). It is hoped that, as more background data accumulate, the black flour beetle (*Tribolium madens*) can be also used in such investigations and the present report is a modest contribution towards that end. There is little information about this species in the literature. Good (1936) notes that it was originally described by Charpentier in 1825 as *Tenebrio madens* and that its present recorded world distribution is western and central Europe, northeast Africa and the United States. In the latter country it is found in certain of the northeastern states, the Great Lakes region, certain of the Rocky Mountain states and the Pacific Coast. It is not reported from South America, Asia and Australia. *Tribolium madens* is clearly less cosmopolitan than either *Tribolium confusum* or *Tribolium castaneum.* Buzicky (1941) discusses its husbandry briefly, and he was well launched on a study of its temperature, moisture and food requirements before his work was interrupted by the war.

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[1] I wish to express my sincere thanks to Dr. Alfred J. Lotka, of the Metropolitan Life Insurance Company, whose advice has been invaluable. I am also greatly indebted to Mr. Albert W. Buzicky, of The University of Minnesota, for furnishing me with a culture of *Tribolium madens* as well as for a number of suggestions. This investigation has been aided by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

The mortality data reported in this paper are obtained in the following way. Two initial cultures are started, one of which contains 125 male imagoes and the other 125 female imagoes. These 250 beetles are within six hours of the same age at the start of the experiment; that is to say, within six hours of eclosion. Each culture container, a standard laboratory finger bowl, is filled with 100 grams of a medium that consists of 95 per cent. whole-wheat flour sifted through number five silk bolting cloth and fortified with 5 per cent. brewers' yeast powder. The two finger bowls are placed in dark incubators at a temperature of 29° C. (with a maximum variation of ± 1°) and at a relative humidity of 60–75 per cent. The containers are not disturbed for five days at which time the medium is poured upon a sheet of paper and the beetles gently removed with a spatula and brush. The number of dead imagoes is recorded and the living forms are returned to their respective dish in 100 grams of fresh medium. This routine is repeated each five days until the last male and the last female dies.

II

The basic biometric constants describing the variation of *Tribolium madens* in respect of duration of life, along with the earlier findings of Pearl, Park and Miner (1941) for *Tribolium confusum* are presented in Table 1. From Table 1 the following points are to be noted:

(1) The mean duration of life for both males and females of *Tribolium madens* is about 220 days or approximately two thirds of a year. This form then, like *Tribolium confusum*, is, for an insect, moderately longevous. Pearl, Park and Miner (1941), however, pointed out that many beetles have a longer average life duration: a conclusion based largely on the findings of Labitte (1916) who reported mean life durations for 21 species (18 genera) of coleoptera. Of these 21, the longest lived form was *Blaps mortisaga* (914.4 days) and the shortest lived form was *Lucanus cervus* (19.16 days). Fourteen of the male representatives of the species and 16 of the female representatives had a mean life duration exceeding *Tribolium madens*, and seven of the male and five of the female a mean duration less than that of *Tribolium madens*.

TABLE 1

BIOMETRIC CONSTANTS FOR LIFE DURATION OF *TRIBOLIUM MADENS* (THIS STUDY) COMPARED WITH *TRIBOLIUM CONFUSUM* (PEARL, PARK AND MINER, 1941)

Species	Sex	Mean (days)	Median (days)	Standard deviation (days)	Coefficient of variability (per cent.)
Tribolium madens	♂♂	199.1 ± 7.64	191.2 ± 9.57	126.7 ± 5.40	63.6
Tribolium madens	♀♀	242.3 ± 7.06	254.5 ± 8.85	117.1 ± 4.99	48.3
Tribolium confusum	♂♂	177.8 ± 2.83	171.6 ± 3.54	84.3 ± 2.00	47.4
Tribolium confusum	♀♀	195.5 ± 3.48	210.7 ± 4.36	103.4 ± 2.46	52.1

(2) The medians do not differ greatly from the means.

(3) Female *Tribolium madens* are longer lived on the average than are males. The mean deficiency in male life duration is 242.3 — 199.1 = 43.2 ± 10.4 days, or 17.8 per cent. of the female mean. This difference is 4.1 times its probable error and may be regarded as statistically significant. It is common experience to find that females

have a greater life expectancy than males. This was true for *Tribolium confusum* and has been reported many times for man (Dublin and Lotka, 1936) as well as for a variety of other organisms (Pearl, 1928). It also agrees with the report of Blunck (1924) on *Dytiscus* and of Labitte (1916) on various other species of beetles. In his tabulation the latter author reported only three cases out of 21 in which males had a greater life duration than females.

TABLE 2

LIFE TABLE FOR MALE TRIBOLIUM MADENS. THE ACTUAL OBSERVATIONS BASED ON 125 IMAGOES

x	l_x	d_x	x	l_x	d_x	x	l_x	d_x	x	l_x	d_x
0–5	125	1	135–140	80	3	270–275	39	1	405–410	7	0
5–10	124	2	140–145	77	1	275–280	38	0	410–415	7	2
10–15	122	4	145-150	76	4	280–285	38	3	415–420	5	0
15–20	118	4	150–155	72	0	285–290	35	2	420–425	5	1
20–25	114	1	155–160	72	1	290–295	33	1	425–430	4	0
25–30	113	0	160 165	71	1	295–300	32	2	430–435	4	0
30–35	113	0	165–170	70	0	300–305	30	1	435–440	4	1
35–40	113	0	170–175	70	1	305–310	29	0	440–445	3	0
40–45	113	5	175–180	69	2	310–315	29	1	445–450	3	0
45–50	108	1	180–185	67	2	315–320	28	3	450–455	3	1
50–53	107	1	185–190	65	2	320–325	25	2	455–460	2	0
55–60	106	1	190–195	63	2	325–330	23	0	460–465	2	0
60–65	105	0	195–200	61	5	330–335	23	0	465–470	2	0
65–70	105	0	200–205	56	1	335–340	23	1	470–475	2	0
70–75	105	1	205–210	55	2	340–345	22	2	475–480	2	1
75–80	104	1	210 -215	53	4	345–350	20	0	480–485	1	0
80–85	103	2	215–220	49	3	350–355	20	1	485–490	1	0
85–90	101	1	220–225	46	1	355–360	19	1	490–495	1	0
90–95	100	4	225–230	45	1	360–365	18	1	495–500	1	0
95–100	96	1	230–235	44	2	365–370	17	1	500–505	1	0
100–105	95	1	235–240	42	0	370–375	16	0	505–510	1	0
105–110	94	6	240–245	42	0	375–380	16	2	510–515	1	0
110–115	88	3	245–250	42	0	380–385	14	1	515–520	1	0
115–120	85	4	250–255	42	1	385–390	13	1	520–525	1	0
120–125	81	0	255–260	41	1	390–395	12	2	525–530	1	0
125–130	81	0	260–265	40	0	395–400	10	2	530–535	1	1
130–135	81	1	265–270	40	1	400–405	8	1	535–540	0	..

(4) Under the conditions of these experiments *Tribolium madens* lives longer than *Tribolium confusum*. The mean difference between the males of the two species is 21.3 days and between the females, 43.8 days. The difference for males is not clearly significant, since it has the borderline probability of 8 per cent. The difference for females is decidedly significant (P = < .07 per cent.). It cannot be definitely concluded whether the greater length of life of the black flour beetle is the result of genetic (species) or environmental factors. In terms of the latter, the two major experimental differentials be-

tween this study and that of Pearl, Park and Miner are the type of medium and the temperature. In the *Tribolium confusum* case the medium was a patent flour rather than the whole wheat-yeast mixture and the mean temperature was 28° C. rather than 29°.

(5) The differences in variability between both sexes and species are not striking and present no consistent pattern.

The mortality data for *Tribolium madens* are presented in two sets of life tables. The first set (Tables 2 and 3) is based on the actual observations of the 125 male and 125 female imagoes. These tables consist of three standard columns: x, age stated as five-day intervals; l_x, the number alive at the beginning of the age interval, and d_x, the number dying during the age interval. The second set of tables (Tables 4 and 5) is computed on the conventional radix of 1,000 rather than 125. In addition to the x column, which is here a 20-day instead of a five-day interval, and the l_x and d_x columns, two other columns are added to these tables. These are the $1{,}000q_x$ column show-

TABLE 3

LIFE TABLE FOR FEMALE TRIBOLIUM MADENS. THE ACTUAL OBSERVATIONS BASED ON 125 IMAGOES

x	l_x	d_x	x	l_x	d_x	x	l_x	d_x	x	l_x	d_x
0–5	125	0	130–135	104	5	260–265	58	0	390–395	18	1
5–10	125	0	135–140	99	2	265–270	58	0	395–400	17	1
10–15	125	3	140–145	97	1	270–275	58	0	400–405	16	2
15–20	122	1	145–150	96	3	275–280	58	0	405–410	14	2
20–25	121	1	150–155	93	0	280–285	58	4	410–415	12	1
25–30	120	1	155–160	93	1	285–290	54	8	415–420	11	3
30–35	119	0	160–165	92	3	290–295	46	5	420–425	8	2
35–40	119	1	165–170	89	0	295–300	41	8	425–430	6	1
40–45	118	0	170–175	89	0	300–305	33	1	430–435	5	0
45–50	118	1	175–180	89	0	305–310	32	2	435–440	5	1
50–55	117	1	180–185	89	7	310–315	30	1	440–445	4	1
55–60	116	1	185–190	82	4	315–320	29	1	445–450	3	0
60–65	115	0	190–195	78	0	320–325	28	3	450–455	3	0
65–70	115	2	195–200	78	0	325–330	25	1	455–460	3	0
70–75	113	1	200–205	78	1	330–335	24	0	460–465	3	1
75–80	112	0	205–210	77	1	335–340	24	1	465–470	2	0
80–85	112	0	210–215	76	0	340–345	23	0	470–475	2	0
85–90	112	2	215–220	76	1	345–350	23	1	475–480	2	0
90–95	110	1	220–225	75	1	350–355	22	0	480–485	2	1
95–100	109	2	225–230	74	2	355–360	22	1	485–490	1	0
100–105	107	0	230–235	72	0	360–365	21	0	490–495	1	0
105–110	107	0	235–240	72	1	365–370	21	0	495–500	1	0
110–115	107	0	240–245	71	2	370–375	21	2	500–505	1	0
115–120	107	3	245–250	69	2	375–380	19	1	505–510	1	1
120–125	104	0	250–255	67	5	380–385	18	0	510–515	0	..
125–130	104	0	255–260	62	4	385–390	18	0			

ing the number dying in a particular age interval among 1,000 alive at the beginning of the age interval, and the $\mathring{e}_x$ column showing the mean length of life remaining to each beetle alive at the beginning of the age interval. The l_x items for male and female beetles are graphed in Fig. 1. In all the tables there is a considerable degree of fluctuation in the reported values owing to accidents of sampling

TABLE 4

LIFE TABLE FOR MALE TRIBOLIUM MADENS. COMPUTED ON A RADIX OF 1,000 BEETLES, AND TWENTY-DAY INTERVALS

x	l_x	d_x	1,000 q_x (per 20 days)	e°_x (days)
0–20	1000	88	88.00	198.40
20–40	912	8	8.77	196.60
40–60	904	64	70.80	178.40
60–80	840	16	19.05	171.20
80–100	824	64	77.67	154.20
100–120	760	112	147.37	146.40
120–140	648	32	49.38	150.00
140–160	616	48	77.92	137.20
160–180	568	32	56.34	128.00
180–200	536	88	164.18	115.00
200–220	448	80	175.57	115.80
220–240	368	32	86.96	118.60
240–260	336	16	47.62	109.00
260–280	320	16	50.00	94.00
280–300	304	64	210.53	78.40
300 -320	240	40	166.67	76.60
320–340	200	24	120.00	70.00
340–360	176	32	181.82	58.20
360–380	144	32	222.22	48.80
380–400	112	48	428.57	40.00
400–420	64	24	375.00	42.60
420–440	40	16	400.00	42.00
440–460	24	8	333.33	43.40
460–480	16	8	500.00	40.00
480–500	8	0	0	50.00
500–520	8	0	0	30.00
520–540	8	8	1,000.00	10.00

and it is well to remember that the entries are not smoothed in any way but are computed from the actual observations.

An examination of the tables and figure shows that male *Tribolium madens* have a higher death rate than do females during the first 280 or so days of imaginal life. There are some exceptions to this that undoubtedly represent random fluctuations but, in general, it is true. The greater mean life-duration of females is primarily a function of the earlier part of the total life span rather than of later life. This fact is well brought out by the survivorship curves (Fig. 1) from which it is seen that the

TABLE 5

LIFE TABLE FOR FEMALE TRIBOLIUM MADENS. COMPUTED ON A RADIX OF 1,000 BEETLES AND TWENTY-DAY INTERVALS

x	l_x	d_x	1,000 q_x (per 20 days)	e°_x (days)
0–20	1000	32	32.00	242.40
20–40	968	24	24.79	230.00
40–60	944	24	25.42	215.60
60–80	920	24	26.09	201.00
80–100	896	40	44.64	186.00
100–120	856	24	28.04	174.20
120–140	832	56	67.31	159.00
140–160	776	40	51.55	149.80
160–180	736	24	32.61	137.40
180–200	712	88	123.59	121.60
200–220	624	24	38.46	117.40
220–240	600	32	53.33	101.80
240–260	568	104	183.10	86.80
260–280	464	0	0	84.20
280–300	464	200	431.03	64.20
300–320	264	40	151.51	85.20
320–340	224	40	178.57	78.60
340–360	184	16	86.96	73.40
360–380	168	24	142.86	59.60
380–400	144	16	111.11	47.80
400–420	128	64	500.00	32.40
420–440	64	32	500.00	35.00
440–460	32	8	250.00	40.00
460–480	24	8	333.33	30.00
480–500	16	8	500.00	20.00
500–520	8	8	1,000.00	10.00

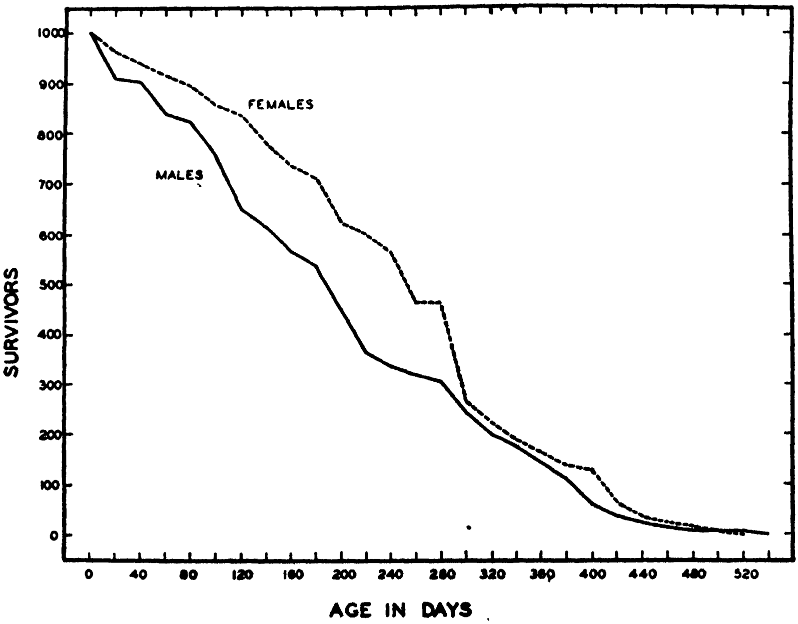

FIG. 1. Survivorship curves for male and female *Tribolium madens* (radix of 1,000 imagoes).

two lines start from the same point, begin to diverge almost immediately, and remain far apart until about 300 days. Thereafter, the differences between them are probably of no great moment. The $\mathring{e}_x$ columns show that the life expectation is consistently greater for females until 220 days, after which there is a strong similarity between the sexes.

Although there are some minor differences in mortality between *Tribolium madens* and *Tribolium confusum,* the basic pattern is fundamentally similar. Both exhibit considerable mortality during early imaginal life (0–60 days) with a steady reduction in survivors thereafter until late in life (380–400 days) after which a few longevous male and female beetles linger on for 100 or so days. In both species the male sex actually has a longer total life span than the female despite the fact that its average duration is decidedly less. It is not certain whether this represents a real sexual difference or is merely a happenstance.

There are some interesting similarities between the survivorship curves of the genus *Tribolium* and those of man. This similarity is more striking perhaps for *Tribolium confusum* than for *Tribolium madens,* but it does exist for the latter species also. The question has been discussed in detail elsewhere (Pearl, Park and Miner, 1941) and the reader is referred to that source if interested.

III

In this paper are presented life tables for males and for females of the black flour beetle *Tribolium madens* Charp., living in regularly renewed flour-yeast medium under favorable laboratory conditions. These mortality data are discussed and compared with those collected earlier for the confused flour beetle *Tribolium confusum.* The latter species along with *Tribolium castaneum* are appropriate, and much studied, organisms for the analysis of experimental population problems. It is hoped that *Tribolium madens,* the subject of this paper, will also prove helpful in this connection, and the object of the present study is to place on record certain basic observations which can be used in later investigations.

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THE RELATIONSHIP BETWEEN SERIAL AND SPECIAL HOMOLOGY AND ORGANIC SIMILARITIES

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INTRODUCTION

IN a critical review of the history of the concept of homology Boyden (1943) takes the view that serial and special homology "have an almost totally unlike significance to the zoologist." He would therefore limit the word homology to Owen's earliest and narrowest definition (which Owen himself later felt compelled to broaden very greatly). Under that original definition homologies exist only between special organs, *e.g.*, vertebrate fore limbs, in a number of different kinds of animals. For the similarities between structures within a single individual, *e.g.*, the fore and hind limbs, Boyden would substitute the old term "homotypy" in place of the commonly used "serial homology." Even such a case as the Drosophila mutant *proboscipedia* in which tarsi-like structures appear on the proboscis does not, according to this view, indicate any serial homology between structures on the same individual, because "the original tarsi still occupy their normal position on the legs."

The contrary view, that there is but one kind of homology of which both serial and special homology are merely aspects, has been strongly supported by Bateson (1894) in his work on variation, and by Hubbs (1944) in his critique of homology and analogy. Hubbs points out that once you admit the homology between any scale *x* of a trout and any scale *y* of a salmon, and between this scale *y* of the salmon and scale *z* of the trout, then it is logically impossible to deny a homology between scales *x* and *z* on the trout. As Bateson put it, "No one has proposed to homologize single leaves of one tree with single leaves on another; it is not expected that the separate teeth of a Roach have definite homologies with separate teeth of a Dace. . . . But in a series whose members are differentiated from each other the existence of such individuality is nevertheless assumed."

HOMOLOGY IN MORE AND IN LESS SPECIALIZED ORGANISMS

The possibility then presents itself that the difference in viewpoint just indicated is largely a reflection of the fact that some investigators focus their attentions chiefly on highly specialized organisms like the mammals or the insects, while others look at less specialized groups. In mammals a one to one correspondence between many special structures appears very evident. All mammals (with nevertheless two or three exceptions) have exactly seven cervical vertebrae. Thus a one to one correspondence between them seems plausible. But even here, if other groups of vertebrates are included, insurmountable difficulties arise. For example, in the words of Bateson, "Which vertebra of a Pigeon, which has fifteen cervical vertebrae, is homologous with the first dorsal of a Swan which has twenty-six?"

Among less specialized organisms Boyden himself comes very close to agreeing that only an arbitrary distinction can be made between special and serial homology. In his consideration of tapeworms Boyden holds that, "Whether the corresponding organs of each proglottid are homologous or homotypic depends on whether one views the tapeworm as a colony or as a single individual." An even clearer case, if that be possible, is to be found in the various freshwater Oligochaetes that reproduce by fission. In a worm like Dero or Nais it is possible to equate a nephridium or any other organ of a given segment, say the fifteenth, with the same organ of the fifteenth segment of another worm. However, when one of these worms reproduces by breaking into two, it usually happens that the nephridia that were in the fifteenth segment of the original individual come to lie in one of the anterior segments, say the sixth, of one of the two new worms. Furthermore, it is possible to vary at will, by controlling the temperature and food supply, the segment of the new individual in which the old nephridia will be.

Thus conditions can be so arranged that the organs in the fifteenth segments of several worms will come to lie, after fission, in the sixth, eighth and ninth segments of the new worms (Van Cleave, 1937).

Two questions at once arise. Is an organ, originally in the fifteenth segment but now in the sixth, homologous with itself of an hour or so earlier when it was in the fifteenth segment? Secondly, are two similar organs, originally in the fifteenth segments of two different worms, no longer homologous with each other now that one has come to be in the sixth and the other in the eighth segment of the new individuals? Perhaps answers can be given to these questions and a clear distinction still maintained between serial and special homology. But from the standpoint of developmental mechanics the questions themselves present no puzzle because they are seen to be essentially meaningless. Before considering the developmental point of view, it should be mentioned that comparable questions arise concerning the relationships of the tentacles in medusae and polyps of the same species of Coelenterate, or the relationships between various structures in Siphonophores, or in the various generations of a single species of Trematode.

HOMOLOGY AND DEVELOPMENTAL MECHANICS

The findings of developmental mechanics are discussed briefly by Hubbs (1944) who throws out the suggestion that these considerations add to the "difficulties" and "complications" of the situation. However, viewed from a slightly different angle, it seems even truer that the concepts of developmental genetics and experimental embryology (if these are indeed not the same thing) simplify rather than complicate these problems.

All will agree that every organism and every structure within an organism is the resultant of the action and interaction of the genes and their products within a particular environment. Under ordinary circumstances the dominating environment for any cell in a developing animal or plant is all the other cells of the embryo, in other words, the organizers of the first and second orders, the fields, gradients, hormones and other influences that may arise as a result of the action of the gene complex of the zygote. Furthermore, the constellation of gene activities primarily concerned with the formation of limbs or any other structures will produce one end result in one developmental environment and some other end result, more or less different, in some changed developmental environment. Likewise, when the developmental environment remains essentially unchanged while one or more of the specific genes becomes altered, a somewhat different end result will be produced. In general, similar developmental forces produce similar end results.

On this basis both serial and special homologies as well as the general homologies seen in feathers, scales and leaves are all the expressions of similar developmental forces. Sometimes these similar developmental forces have acted within several different kinds of animals or plants, sometimes within different parts of a single individual. Exceptions to this rule will be discussed later, but this is apparently true of the great mass of similarities between organisms to which the word homology is usually applied. In phylogenetically related species, all homologies, serial and special alike, go back to ancestral similarities; ultimately to identities, with possible exceptions to be noted later.

To illustrate with a familiar specific case, all the appendages of the ancient Trilobites were biramous and identical or nearly so, from head to tail. According to the view outlined above, all the appendages were anatomically the same because they were all due to the same developmental causes. So to-day all the nephridia of an annelid or the scales of certain fish or the leaves of trees are alike for the same reason—similar developmental causes. During the evolution of the Arthropods from the Trilobite-like ancestor into all the various species of the modern Crustacea several possibilities present themselves. Genes may have changed in such ways that it then made a difference whether the limb primordium developed at the anterior or the posterior end of the animal or at some intermediate level. Consequently, the appendages found at the different levels of the body came to differ from each other. In different lines of descent these changes must have been different. Thus different species have appeared with appendages modified in different ways. One and the same process, in fact one and the same mutation can thus have led simultaneously to the differences that are recognized between serially homologous and specially homologous structures. In any specific case it may have been gene activities chiefly concerned with the development of the limb anlagen that underwent most of the changing, or it may have been the gene activities primarily concerned with the general embryonic environment. Over a period of time both were probably involved.

Developmental forces that would fit into a picture of this kind are well known. A large proportion of the known gene mutations appear to affect some particular structure in a highly localized and specific manner. The eyes, wings, horns or perhaps particular bristles are changed while all the rest of the organism seems at least to be untouched. But a great many gene changes have been observed that are general in their effects. Such gene changes alter the growth of hair or feathers on every part of the body, change the amount of pigment deposited throughout the organism or are responsible for other results not limited to a localized structure. Turning to directly observable developmental forces, there are the ubiquitous metabolic gradients so long studied by Child and his school. Cases are known in Amphibia where head ectoderm possesses very different developmental potencies from ectoderm taken from more posterior regions. For example, gill-forming mesoderm will form a gill if overlain with any head ectoderm. If it is covered with ectoderm from flank, the formation of a gill is suppressed (Severinghaus, 1930).

The theory developed by Goldschmidt (1940) to account for homeosis in Drosophila also fits into this scheme nicely. In the case of *aristopedia* in which the antennae are replaced by the tarsus of the leg, it had been found by Balkaschina (1929) that normally the imaginal discs for the legs develop and differentiate before those for the antennae but that in *aristopedia* the imaginal discs for the antennae develop at the same time as those for the legs and develop as legs from the beginning. Goldschmidt's explanation runs approximately as follows. The anlagen or imaginal discs for all the appendages are at first identical in potency. The kind of structure each develops into depends on the embryonic environment at the time of its development. The developmental forces, probably largely determining substances, within the embryo change with a definite velocity and in a definite order during the course of embryogenesis and later development. All imaginal discs that develop simultaneously are in a plastic condition at the same time and therefore come under the same determining influences and develop into the same structures. Any influence that changes the rate of development of an imaginal disc automatically changes the developmental forces presiding over its differentiation and consequently changes the ultimate structure formed. Presumably it would also be possible that some change could take place in the nature of the discs so that they would respond or fail to respond in new and different ways to the same sequence of developmental conditions.

A simple but illuminating situation is found in the Siamese cat and in the Himalayan rabbit (Iljin, 1931). In these animals all the hairs are light tan (in the cat) or white (in the rabbit) if they develop at any point on the skin where the temperature is above a certain critical level. Wherever the skin temperature falls below that level, as it does on the tips of the ears, nose, feet and tail, the hairs that develop are black. Experimentally it is possible to increase the area of black hairs until it covers the entire body by allowing the hairs to grow out at abnormally low temperatures. Likewise the area of tan or white hairs can be increased by raising the temperature of the room in which the animal lives during hair growth. Similar changes in the area of pigmentation can be produced at will by raising or lowering the thyroxin level in the animal. It is possible to imagine related species in which the skin all over the body is above the critical threshold temperature and which is consequently completely white, or a related species in which the skin for some reason, perhaps hormonal, is everywhere below the critical level and which is consequently all black. Clearly, from the developmental point of view, most of the similarities or homologies, whether between structures in different species or in different parts of the same species, *i.e.*, whether special, serial or general, are all similar because due to similar developmental forces.

One of the results of this concept of homology is that it renders it unnecessary, in fact meaningless, to talk about "moving" the tarsi or any other structures from one level of the body to another. When the total area of black hairs is increased by allowing the hairs to develop in the cold, black hairs are not "moved" from the tips of the ears to their bases. Obviously enough, the developmental conditions have so changed that in respect to pigment formation the situation at the bases of the ears has become like that at their tips. Just so when tarsi appear in the mouth region, or the eye becomes an antenna, it is irrelevant to ask whether or not the "original" tarsi or the "original" antennae are still in their usual positions. If the tarsi are still found in their usual place, that would merely indicate that the change which brought about their development in a new location was not accompanied by some condition which inhibited their development in the old. Contrariwise, if the tarsi are no longer present in their old site, it merely indicates that the change was accompanied by some inhibitory factor.

It is tempting to compare what is now known about the interaction of developmental forces with Owen's theory that the form of any animal is the result of the interplay of what he termed an "all-pervading polarizing force" and a "special organizing force." Doubtless there is a certain rather vague correspondence between these concepts and present ideas about primordia, fields, gradients, organizers and gene action, although Owen lacked all the concrete information now available. Certainly if there is to be any return, as has been suggested, to Owen's ideas, it will have to be to his often neglected suggestions of developmental forces and to his later and most general conception of homology rather than to his first and narrowest.

HOMOLOGY AND SIMILARITY

It is important to remember that the general principle stated at the beginning of the previous section, *i.e.*, from the developmental point of view all homologies whether special or serial are the expressions of similar developmental forces acting sometimes in different kinds of animals, sometimes in different parts of the same animal and tracing back to ancestral identities, is a principle that is only true among related organisms and not always true even there. Moreover, the term developmental forces is such a broad term that it demands further definition. As de Beer (1938) has made quite clear, "characters controlled by identical genes are not necessarily homologous" and likewise "homologous characters need not be controlled by identical genes." Among other examples he cites a single gene in poultry that controls both the formation of a crest of feathers and the formation of a cerebral hernia. Despite the fact that they are both controlled by a single gene, no one would suggest a homology between two such diverse structures. De Beer also cites the way eyes may be made to reappear in homozygous "eyeless" stocks by reshuffling the gene complex in such a way that other genes can "deputize" for the normal allele of the "eyeless" gene. Since even when homologous characters are identical in structure they need not be controlled by identical genes, de Beer concludes, "it is likely that when homologous characters do not resemble one another closely the genes controlling them may be very diverse." Of course a certain amount of diversity must exist or the resulting developmental forces and consequent final structures would be identical.

In the end the concept of homology will probably undergo the same kind of development that many other scientific concepts have undergone. As every one knows, originally all differences from the norm for a group were lumped together simply as "variations." Now it is clear that variation includes at least two very different kinds of events—hereditary and non-hereditary variations. In a similar way homology may continue to be a useful word to denote correspondences between different organisms or different parts of the same organism. But with the growth of genetical and embryological knowledge it is becoming increasingly evident that the homologies or, better, the similarities between organisms or their parts are of several different kinds.

Perhaps it would be well to discard the word homology altogether and to substitute the neutral descriptive term, similarity. Is not "homology" something of a straw man? A group of calendar reformers who set out to ask how many days there "really" are in a week would obviously be ridiculous. Are biologists who ask what homology "really" is, very different? At best homology means some kind of similarity, and of organic similarity there are various kinds.

Among organic similarities the basic distinctions are not between serial, special and general homologies because these in fact can not be distinguished unless attention is focused on a few limited cases. The basic distinctions among the similarities of plants and animals would rather seem to be those based on (1) developmental, (2) genetical and (3) evolutionary differences. Thus some similarities are the result of similar developmental forces, while other similarities (probably much rarer) are the result of quite different developmental forces. The former may be termed homodynamic, the latter heterodynamic similarities. Some similarities are the result of variations on a common basic gene complex, while other similarities arise as "convergent" variations on radically different gene complexes. These may be called homogenetic and heterogenetic similarities respectively. For the similarities that trace back to a common ancestor the word homophyletic suggests itself; for those that do not trace back to a common ancestor the word heterophyletic. Consequently, with three pairs of alternatives there are eight theoretically possible categories of similarities.

Most of the similarities to which the word homology is commonly applied are apparently homophyletic, homogenetic and homodynamic. Here belong the familiar similarities between the limbs of vertebrates or the scales of fish. For a tremendous number of cases, however, not enough is yet known about the evolution, the genetic basis or the embryological factors concerned to be able to say with any assurance what kind of similarity is present. For example, this is certainly true of the concentric circles of pigment that are so frequently found in the wings of butterflies and moths, the feathers of birds and the skin of fish, salamanders, and reptiles. A like ignorance surrounds the striking similarities between the eyes and also the mouth parts of certain molluscs and vertebrates. Some of these instances, especially the concentric rings of pigment, would appear at least to be due to closely similar developmental forces, *i.e.*, to be homodynamic. At the same time, since they are in such very diverse kinds of animals, most of these cases probably arise from very different gene complexes. Most certainly, in the case of the butterflies and the fish, the similarities are not historically connected in the actual course of evolution. Consequently, these particular likenesses would seem to be homodynamic but heterogenetic and heterophyletic. All this is of course as yet speculation.

CONCLUSION

As Bateson and Hubbs have both demonstrated, the distinction between special, serial, and general homology is not a valid one. This becomes clear as soon as attention is shifted from the limited cases found in highly specialized organisms to the more general situation represented by the scales of fish or the vertebrae of birds. From the standpoint of developmental mechanics, most of the similarities whether called special, serial or general homologies are all alike the end result of similar developmental forces acting sometimes in different parts of a single individual, sometimes in individuals of different species. Very often these similarities trace back to ancestral identities.

The word homology had perhaps best be discarded as too charged with metaphysical overtones and a neutral descriptive term like similarity substituted.

The basic distinctions between the similarities shown by animals and plants would seem to be those based on (1) developmental, (2) genetic and (3) evolutionary differences. Similarities due to similar developmental forces may be called homodynamic, those arising from different developmental forces, heterodynamic. Similarities arising out of variations on a common gene complex may be termed homogenetic, those arising from radically different gene complexes, heterogenetic. Likewise any similarities that are derived from a common ancestor may be called homophyletic, those not so derived, heterophyletic. Thus eight different categories of organic similarities are theoretically possible.

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ON PARTIAL MELANISM ASSOCIATED WITH PARATHYROID ENLARGEMENT IN PIGEONS

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MELANISM arising from physiological disturbance has not previously been reported for domestic pigeons. A few cases of partial melanism have been observed in the pigeon colony of this department under circumstances which suggest the nature of the factors intimately associated with the belated appearance of this condition. It has been found that, on the diet supplied to birds of this colony, a deficiency of sunlight regularly leads to enlargement of the parathyroids and also seems to be concerned in the occasional onset of partial melanism. Since we do not plan a further study of this type of melanism it seems useful to record these observations in connection with data showing the relation of sunlight to parathyroid enlargement and rickets in pigeons.

Black pigeons are of course quite common, and this and other hereditary types which might be mistaken for "physiological" melanism have been studied fairly extensively (Hollander, 1938); such types are not considered here. Also excluded are genetic mosaic effects (aberrantly colored patches of plumage or black flecks and streaks in feathers or piebaldness) which are fairly well understood (Hollander, 1944). The melanism reported here had the following characteristics: (*a*) it was observed only in adult female pigeons of essentially wild-type coloration—gray-blue with black bands on tail and wings; (*b*) it was definitely partial rather than complete melanism; (*c*) it first appeared after one or more moults; (*d*) when the blackening did not involve entire feathers it produced transverse bands on the feathers, not longitudinal streaks typical of mosaic effects.

Cases of Melanism

The first specimen, E149, was noted to have melanistic feathers scattered among gray-blue ones when she came to autopsy at the age of 4 years (Fig. 1, *C*). This bird had been breeding, though not very successfully, and for some weeks had been ill; one wing was lame, probably from a disease (paratyphoid) which commonly produces this condition. The only unusual finding at autopsy was a yellow tumor-like body (weighing approximately 50 mg) adjacent to the right thyroid gland. This body was sectioned (by Miss V. M. Rauch) and identified as parathyroid tissue.

Four other domestic pigeons showed melanistic feathers, more particularly on the rump and breast. Two of these cases (Nos. X566 and X177) have been killed. No. X566, aged 16 months, had been ill and emaciated for some weeks; the parathyroids were not weighed but were rated as three to four times normal size. No. X177 was killed at 30 months in apparently healthy condition; the four parathyroids weighed 16 mg, and the autopsy showed no other marked deviation from normal. The other two females, X803, X899, are still alive (Fig. 1, *A* and *B*); they were biopsied when 12 and 13 months old, respectively, and their parathyroids were estimated at about six and four times the normal weight. The three females last described had remained in fair or good health; they had produced fair numbers of eggs and their bones were not obviously softened. All the melanistic pigeons were kept and bred in cages with exceptionally little exposure to light during many months before the melanism was observed. Although a glass side of their colony house was removed during the summer, these birds were then exposed to very little direct sunlight.

An informative though indecisive test was made on two of the melanistic pigeons (X803 and X899) listed above. Immediately following the biopsies on their parathyroids these birds were fed a single capsule of concentrate (1800 I.U. of vitamin D; 15,000 I.U. of vitamin A) of cod-liver

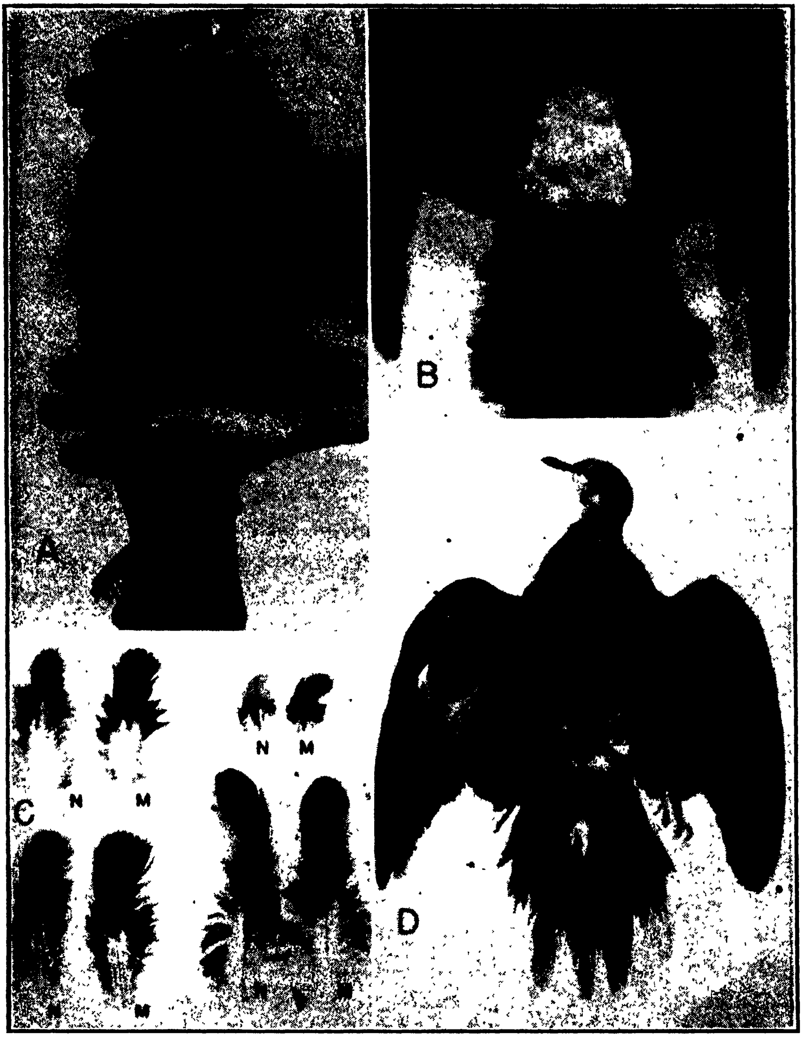

FIG. 1. A. Ventral view of ♀X899. Most of the visible breast plumage shows partial melanism; the patch of normal plumage on the anterior left breast of the bird was grown following administration of cod-liver oil concentrate. B. Dorsal view of ♀X899 showing many melanistic feathers. C. Pairs of adjacent normal (n) and melanized (m) feathers plucked from ♀E149. D. Dorsal view of preserved skin of hybrid dove, ♂D918, showing scattered melanistic feathers, especially in left-wing coverts.

oil. The new breast feathers which grew over the site of the operation were not melanistic (Fig. 1, *A*). Since no control is available one must admit the possibility that the oil (vitamins D and A) was not responsible, but the result does conform with the view that this type of melanism is connected with vitamin-D (light) deficiency.

A sixth case of melanism occurred in a hybrid dove (D918) (derived from *Zenaida vinaceo-rufa* and *Zenaidura carolinensis*). This male dove was very old (18.1 years) at death. During at least the final five years of its life it was unable to fly, and some melanism was present during much or most of this period. Some black spots, restricted to certain feathers of the wings, are normal in these birds. For at least two years before its death some black feathers were scattered throughout its plumage along with other feathers of normal or nearly normal color (see Fig. 1, *D*). This bird was exposed to some direct sunlight during summers only; its inability to fly, however, forced it to live on the floor of its cage where light was most restricted. At autopsy the parathyroids of this small (140 gm) bird weighed approximately 18 mg.

PARATHYROIDS AND RICKETS

During recent years parathyroid enlargement has been observed rather frequently in pigeons of this colony, more especially in squabs with pronounced rickets and occasionally in adult breeding females with osteomalacia. In such pigeons the parathyroids sometimes weighed almost as much as the thyroids; values of 20 to 35 mg (total for 4 glands) were not rare. Deficiency of vitamin D and sunlight almost certainly induced this enlargement of the parathyroids and the frequently associated defective ossification of the bones. The occurrence of enlarged parathyroids and soft bones was known to be limited to birds reared or kept in cages where little or no ultraviolet light entered. The diet of those birds was not fortified with vitamin D.[1] On the same diet, and with direct sunlight, no pigeons have been found with parathyroid enlargement.

Within the past year several of these birds with soft bones have been killed at various stages of immaturity. The weight of the parathyroids of these birds was obtained, and records made of the light conditions in the cages in which such birds were reared. Similar weights and records were obtained for other birds which, though reared under similar conditions of lighting, showed no obvious softening of the bones. Still other normal young, hatched and reared in cages exposed daily to some hours of direct sunlight (after removal of glass sides from the colony houses on May 10), were killed (July, August) in order to obtain adequate control data for both of the preceding groups. Data obtained from those three types of

TABLE 1

WEIGHTS OF PARATHYROIDS OF PIGEONS IN RELATION TO DEFECTIVE OSSIFICATION AND DEGREE OF EXPOSURE TO DIRECT SUNLIGHT

Condition of keel and femur	Age	Degree of exposure to sunlight	Date	Sex and number	Weight of parathyroids			Body weight avg.
					Minimum	Maximum	Average	
	mos.				mg.	mg.	mg.	mg.
Soft	2.8	Slight	4/27	♂♀6	20.3	35.0	28.5	239
Normal	2.7	Slight	5/8	♂♀7	5.0	10.1	7.4	298
Soft	2.0	Slight	5/15	♀3	16.0	18.4	17.2	228
Normal	3.1	Slight	5/15	♂3	6.9	9.7	8.5	320
Soft*	1.3	Slight*	5/6	♂♀3	8.8	17.4	12.3	(200)
Normal	1.1	Slight	6/21	♂1			5.9	230
Normal	2.0	Complete	7/25	♂♀6	1.8	4.3	3.4	321
Normal[1]	23.5	Complete[1]	8/8	♂♀5	3.4	4.2	3.7	390
Normal[2]	2.7	Complete	8/15	♂♀11	1.7	4.8	3.3	(430)
Soft keel[3]	31.0	Slight	7/25	♀1			27.8	251

* Young and parents both subjected to a low calcium diet (corn, Kafir and salt; no grit except sand) and to no direct sunlight.

[1] During the previous 3 months these birds were exposed to direct sunlight during several hours daily.

[2] Birds of a large breed (Carneau) injected daily for 15 days (dihydrotachysterol, estradiol or fed aluminum hydroxide) before measurement.

[3] Apparently a case of acute osteomalacia, but femur nearly normal; killed 3 days after laying egg.

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[1] The chief source of traces of vitamni D in the feed would probably be Kafir (or milo). These grains ripen under partial exposure to sunlight. The other ingredients of the diet were cracked yellow corn, wheat and peas. The commercial grit kept before these birds contained oyster shell, calcite, sand, salt, charcoal and Venetian red.

young are given in Table 1. Some data for adults are included in the tabulation. It will be observed that the weight of the parathyroids of either young or old pigeons which had been exposed daily for some months to direct sunlight ranged from 2 to 5 mg. Young reared in relatively dark cages (and behind glass) showed two grades of parathyroid enlargement. Those with soft keels and femurs had extremely enlarged parathyroids, while those without obvious bone defects had parathyroids only about double normal size. However, one squab reared in the sunlight on a calcium-deficient diet developed severe rickets with little or no parathyroid enlargement, while three other young on a calcium deficient diet in the absence of direct sunlight showed some enlargement of the parathyroids (Table 1).

DISCUSSION

Higgins and Sheard (1928) reviewed the earlier literature and also carefully tested the relation of light rays and vitamin D deficiency to parathyroid enlargement in chickens. They concluded that vitamin D deficiency causes the parathyroid enlargement. Oberling and Guerin (1934) later reported, on the basis of evidence very inadequately described, that not vitamin D but a mineral deficiency is the cause of parathyroid enlargement. Doyle (1925) described rickets in mature laying fowl and noted its association with parathyroid hyperplasia. His description of the rachitic symptoms in adults fits well the single case (acute osteomalacia?) tabulated by us; it also fits several additional cases which we observed without obtaining weights of the parathyroids. Hughes, Payne and Latshaw (1925) showed that either sunshine, ultra-violet radiation or the feeding of cod-liver oil prevented the appearance of leg weakness (rickets) in young fowl.

Witschi (1940) has briefly noted, without giving data or providing comment, that he had observed cases of melanism in the weaver finch associated with "a peculiar type of parathyroid hyperplasia (unpublished)." The finches were caged in the laboratory and presumably received little direct sunlight and no viamin D supplement in the ration. The older literature on melanism in caged wild birds (Beebe, 1907; Rensch, 1925) is not explicit as to exposure to sunlight but it may be assumed that the birds described had received little or no direct sunlight, especially in winter. The parathyroids are not mentioned in these older studies. Beebe (1907) attributed melanistic changes in caged Inca doves (*Scardafella inca*) and finches to humid environment, and apparently extended that explanation to the several cases of melanism in caged birds which he found in the literature. It now seems more probable that the cases observed by Beebe, like our own cases, involved parathyroid disturbance and deficiency of vitamin D.

No comparable melanism has been reported in poultry despite the fact that most observations on vitamin-D deficiency and parathyroid hypertrophy have been made on fowl. This may be due largely to the use of genetically white varieties for these studies, but it is also possible that certain types or shades of color do not become melanistic under vitamin-D deficiency. On this point we note the remarkable fact that the blond ring doves of this colony have shown no clear case of melanism although some thousands of this species have been observed to or beyond the third year of life. These doves were offered the same diet as the *Zenaida* hybrid (above), but a diet that was different from that of the pigeons. The diet of doves contained hemp seed, Kafir or milo, wheat, cracked corn, canary seed and millet.

Rachitic squabs never have shown melanism even when their parathyroids were very large. A more prolonged deficiency, or an element of aging, therefore seems to be necessary for the appearance of melanistic feathers. Something more than chance is also probably responsible for the fact that this type of melanism has not been observed in a male pigeon. On this point it is notable that, unlike the adult female, the male's supply of calcium and vitamin D is not subject to the special drains and losses which necessarily accompany egg production.

SUMMARY

Five cases of non-genetic partial melanism were observed in adult female domestic pigeons. One similar case was observed in an old male hybrid (*Zenaida* × *Zenaidura*) dove after it became unable to fly.

Two physiological conditions, slight exposure to sunlight and enlargement of the parathyroids, were known to be associated with all of these six cases of melanism. It is considered probable that these conditions were also responsible for previously reported instances of melanism in caged wild birds. In two instances plucked melanistic feathers were replaced by normal feathers following the administration of a concentrate of cod-liver oil (vitamin D).

Parathyroid enlargement is shown to occur regularly in young pigeons reared on a mixed grain diet in the absence of direct sunlight (vitamin-D deficiency). Grossly defective ossification of the bones (rickets) of many such pigeons is reported.

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REVIEWS AND COMMENTS

EDITED BY PROFESSOR CARL L. HUBBS

IN these reviews and notices of current biological publications emphasis is given to books and major articles which fall within the special scope of THE AMERICAN NATURALIST, in that they deal with the factors of organic evolution. REVIEWS AND COMMENTS are meant to include also such general discussions, reports, news items and announcements as may be of wide interest to students of evolution. Except as indicated, all items are prepared by Dr. Carl L. Hubbs, Scripps Institution of Oceanography, University of California, La Jolla, California. All opinions are those of the reviewer.

Configurations of Culture Growth. By A. L. KROEBER, Berkeley: University of California Press, 1944: i–x, 1–882, 8 charts and 1 map. $7.50.

THE amount of historical research that Kroeber expended in this study was matched only by the breadth of his competence. The result is an outstandingly scholarly contribution to the analysis of patterns in the evolution of human culture, as this has been expressed throughout the world in philosophy, science, philology, sculpture, painting, drama, literature, music and the growth of nations. The emphasis is on the configurations of culture in time, in space and in degree of development, rather than on the content, quality and causes of different cultures. The point of view is that of an eminent anthropologist, definitely not that of a biologist. Almost no credence is given to any genetic basis for the differences with time in the degree of cultural development. Genetic changes within human groups having one culture are not categorically denied, but are interpreted as having been of little significance in the growth of cultures. Potential eminence of individuals is regarded as relatively invariable with time, as perhaps also with race. Genius, which is adopted as the index of cultural development in any intellectual field, is held to be suppressed more often than it is realized. The suppression and the realization are attributed primarily to environmental factors.

In his non-biological approach, which will prove disappointing to some, the author seldom presents his conclusions in terms of evolutionary principles. In fact he seems to avoid even the term cultural evolution, writing, instead, "the configurations of cultural growth." On reading even extracts from the large volume, however, there will occur to a modern evolutionary biologist many correspondences—analogies if you wish—between the generalizations which Kroeber reaches and the concepts of speciation and phylogeny. The more one considers such common principles the more may he become impressed how much there is in common between the laws that regulate the different sorts or levels of evolution.

That potential genius and the cultural developments which might have resulted from pre-eminent minds are ordinarily unrealized through environmental suppression is an assumption which strikingly agrees with a modern evolutionary concept. According to this concept evolution is ordinarily retarded by reason of biotic saturation, and becomes rapid or even explosive when the environment is unsaturated. The idea that geniuses tend to occur more or less contemporaneously, so as to produce peaks of culture, corresponds to the evidence that organic evolution operates by spurts, conditioned by the decline of groups previously dominant or by the availability of new environments. That like cultures evolve repeatedly from the same or similar cultures reminds one of the parallel evolution which results when the evolutionary stage is set—for instance, the repeated origin of viviparity in the cyprinodont fishes, which swim together at the surface in waters that often become so deficient in dissolved oxygen as to endanger the developmentary stages. That like cultures also arise in response to similar conditions may be looked on as an expression of the general principle of convergent evolution. That cultures may persist for long periods in a retarded condition parallels the idea of suppressed evolution. That cultures tend toward bizarre modifications following the peak of their development and preceding the period of decadence reminds one of evolutionary "overspecializations." Cultures as well as groups of organisms may become extinct either through replacement or through hybridization. Cultures as well as groups generally diffuse from a center of origin. As an inexact and unreliable approximation, the area occupied by either a culture or a group is positively correlated with the age of this culture or group. Environment is a factor of profound importance in both cultural and organic evolution.

In several respects present-day culture is allocated in the decadent stage. This judgment will no doubt be challenged by some. And at this moment one can only wonder what may be the "configurations of culture growth" during the Age of Atomic Energy.

NOTICES OF NEW BOOKS AND ARTICLES

The Influence of Types of Soil upon the Local Distribution of Some Mammals in Southwestern Utah. By ROSS HARDY. Ecological Monographs, 15, 1945: 71–108, figs. 1–19.—Soil texture is shown in this thorough study to be a significant factor directly influencing the local distribution of small burrowing mammals in an arid region. Concentration of soil chemicals and soil moisture act chiefly through the effect of these factors on the vegetation. Other conditions involved in determining the presence or absence of certain types of mammals are the depth and the slope of the soil. The color of the soil affects the distribution of light and dark races.

In agreement with the conclusions by Dice and other workers soil color is indicated as a speciational factor of importance. Where soils of a certain color occur with consistency over fairly large areas, the mammals inhabiting these areas tend to approach the soils in depth of pigmentation and in quality of color. Such speciation in the region studied by Hardy seems to have been accentuated by the presence of an effective distribution barrier, the Beaverdam Mountains, lying between areas where the soils are predominantly different in color.

Weeds of Lawn and Garden. A Handbook for Eastern Temperate North America. By JOHN M. FOGG, JR. Philadelphia: University of Pennsylvania Press, 1945: i–vii, 1–215, 175 figs. $2.50.—This pocket size handbook by the University of Pennsylvania's able botanist, Professor John M. Fogg, Jr., should be of real value to the gardeners in the section of the country east of the Mississippi River. In a brief but informative introduction the author discusses the general characteristics of weedy species, the geographic origin of weeds, the extermination of weeds, chemical control of weeds and weed ecology. The illustrations are excellent. Amateur gardeners should have no difficulty in identifying a particular plant either in the juvenile or mature stages. There is a glossary, a good index and a bibliography. One wonders why the comprehensive text, "Weed Control," by Robbins, Crafts and Raynor, has been omitted from the bibliography.—THOMAS W. WHITAKER, *U. S. Department of Agriculture, Box 150, La Jolla, Calif.*

Green Cargoes. The Story of the Transportation of Seeds and Plants from Their Original Homes to the Four Corners of the Earth. By ANNE DORRANCE. Garden City, New York: Doubleday Doran & Co., Inc., 1945: 1–187. $2.50.—This book represents an attempt to recount in popular style the history of plant introduction both ancient and modern. In her interpretation of the results of recent research the author appears to have been unnecessarily dogmatic. Mangelsdorf and Reeves would be the last to claim that they had found the complete answer to the origin of corn. At times there is an uncertain mixture of fact and fiction. For example, corn investigators will be surprised to learn that wind blew the seed of maize southward to the Andes and northward to Mexico. There is a limited bibliography. The book might be of interest to gardeners and horticulturists.—THOMAS W. WHITAKER, *U. S. Department of Agriculture, Box 150, La Jolla, Calif.*

Plant Geography and Culture History in the American Southwest. By GEORGE F. CARTER. New York: Viking Fund Publications in Anthropology, 5, 1945: 1–140, figs. 1–27. $1.50. —This monograph attempts to correlate the domestication and subsequent distribution of the important crop plants of the Southwest with the archeological history of this region. On the basis of an extensive ethnological survey, involving two seasons of field work, and a minute sifting of pertinent botanical and archeological literature, the author has built up a credible picture of the interrelations of domesticated plants and cultural history. Although this is probably not the first time that a union of the two disciplines, botany and archeology, has been used to unravel cultural history, it seems to be the first time the method has been employed so extensively. Admittedly some of the conclusions are tentative, and must await further investigation to establish their validity. However, the author deserves credit for exploring a field that promises to make a worthwhile contribution to both botany and archeology. Plant scientists interested in the domestication of crop plants will find much of value in the sections on cucurbits, maize, tepary bean, kidney bean, and cotton. There is a bibliography of 167 titles, but no index.—THOMAS W. WHITAKER, *U. S. Department of Agriculture, Box 150, La Jolla, Calif.*

The Galapagos Finches (Geospizinae), A Study in Variation. By DAVID LACK. San Francisco: Occ. Pap. Calif. Acad. Sci., 21, 1945: i–vii, 1–159, pls. 1–4, figs. 1–26, 1 map. $2.50.—The Galapagos finches challenge the student of speciation and evolution by reason of the complexity of their variations and the difficulty of ascertaining the structural and physiologic boundaries between species. For Darwin the group posed major problems, but his study of it did not yield an adequate explanation of the origin of its various units; natural selection was too dominant in his thinking. It is only through the application of the "Sewall Wright effect" and of recent advances in knowledge of colonization phenomena in insular birds that Lack has reconstructed a balanced picture of origin which seems to be thoroughly satisfactory.

Hindrances to proper understanding of the group by early workers were inadequate series of specimens and erroneous locality data. Swarth's (1931) detailed taxonomic and nomenclatural treatment largely cleared up the tangle of evidence. David Lack has reviewed essentially the same assemblage of specimens and has proposed some changes in Swarth's taxonomy, but none of these is fundamental. Statistical analysis made by Lack has led to a more conservative attitude in recognizing some weak subspecies; and, in keeping with current trends in ornithology, some closely related geographic representatives have been combined in one species.

The weakest phase of Lack's work is that on sexual dimorphism of plumage and the complicated plumage succession in the age groups within a species. Had the author early possessed a full realization of the intricacies of molt and plumage sequence when reviewing specimens, better data could have been assembled on these topics. For example, one suspects that there has been some confusion of postjuvenal and juvenal plumages, and the reader can not always be sure that the mixed black plumages referred to are static situations at the time the birds were taken or are the result of molt then in progress. These uncertainties have probably not yielded any wrong conclusions but have merely prohibited the formulation of a clear account of progressive attainment of adult male plumage in the individual and the details of evolutionary regression of sexually dimorphic color. In any event, plumage and molt sequence would doubtless have remained imperfectly known pending further field and aviary study.

Important in Lack's analysis of these finches is his personal experience with them in the field where courtship behavior, interspecific contacts and habitat were observed. Breeding habits of the species are essentially the same. In aggressive and courtship behavior the species appear to recognize each other primarily by difference in size and proportions of the bill. It is this structure also which the taxonomist relies on most in separating them. Closely related species occupy extremely similar or possibly identical habitats and they usually eat the same food. The main genera, however, have different feeding habits and show adaptive radiation.

A unified population of any one species of geospizine is not abnormally variable, contrary to prevailing notions held on this subject. Biologically distinct species may be but slightly differentiated in structure, often less so than mainland species. The complexity of the group lies in the extensive local geographic variation of differing magnitudes. Island populations of each species or even race are often slightly different in mensural characters to a degree insufficient to justify subspecific recognition. The dividing point in bill size between two non-interbreeding, closely related species may have different absolute values on different islands.

Birds have not reached the Galapagos Islands *via* a land bridge, although the islands have been variously connected with each other in the past. The small, isolated islands of the archipelago have fewer species of geospizines and a higher proportion of endemic forms than have the larger or the central islands.

"The most important factors in the evolution of the Geospizinae have probably been the almost complete absence of food competitors and predators and the existence of several islands which provide partial, but not complete, isolation for island forms." Differences between island forms of the same species are considered non-adaptive and due primarily to the random fixation of variability; colonization by an atypical example may be a subsidiary factor.

"Species probably originated mainly through geographically isolated races which later met and kept distinct, but some forms may be of hybrid origin." Characters of closely related species, like those of subspecies, are non-adaptive except that bill characters serve in species recognition. "The species of Geospizinae . . . do not show the degree of overlapping and hybridization sometimes claimed for them, and there is no need to assume for them some quite exceptional method of evolution."

This valuable and sound interpretation of a classic case of variation is a credit not only to the author but to the sponsoring agencies and to the editors who, because of the war, have had much to do with the preparation of the report. The instructive value of the Galapagos finches has by no means been exhausted, but the subject has been nicely brought up-to-date by this work.—ALDEN H. MILLER, *Museum of Vertebrate Zoology, University of California, Berkeley 4, Calif.*

Social Darwinism in American Thought 1860–1915. By RICHARD HOFSTADTER. The American Historical Association. Philadelphia: University of Pennsylvania Press, 1944: i–viii, 1–191. $2.50.—In turning to this book an evolutionary biologist is apt to be disappointed in not finding an adequate consideration of comparative sociology. In condemning a biological viewpoint one might expect a naturalistic approach, which, however, is hardly evident. No impact on the author appears to have been made by the conclusions of Ritter and of some other biologists who have held that cooperation is a major factor in the evolution of biotic societies and in the phylogeny of organisms which have their own social organization or which maintain mutual-aid relations with other organisms. Instead of relying on such views (as he might have done) Hofstadter disavows the Darwinian basis of human societal relations.

> There was nothing in Darwinism that inevitably made it an apology for competition by force . . . American society saw its own image in the tooth-

> and-claw version of natural selection . . . its dominant groups were thus able to dramatize this vision of competition as a thing good in itself. Ruthless business rivalry and unprincipled politics seemed to be justified by the survival philosophy. . . . This version of Darwinism depended for its continuance upon a general acceptance of unrestrained competition. . . . In time the American middle class shrank from the principle it had glorified, turned in flight from the hideous image of rampant competitive brutality. . . . While Darwinian individualism declined, Darwinian collectivism of the nationalist or racist variety was just beginning to take hold. Darwinism was being made to fit the mold of international conflict-ideologies. . . . The survival of the fittest had once been used chiefly to support business competition at home; now was used to support expansion abroad. These dogmas were employed with success until the outbreak of the first World War. Then, ironically, the ''Anglo-Saxon'' peoples were swept by a revulsion from international violence. . . . A resurgence of social Darwinism, either in its individualist or imperialist use, is always a possibility as long as there is a strong element of predacity in society. . . . Whatever the course of social philosophy in the future, however, a few conclusions are now accepted by most humanists: that such biological ideas as ''the survival of the fittest,'' whatever their doubtful value in natural science, are utterly useless in attempting to understand society [and so on].

It should be remembered, however, that the qualities that lead to survival in societies and in social organisms may involve cooperation and mutual aid, as well as struggles unto the death.

The Marine Annelids of North Carolina. By OLGA HARTMAN. Duke Univ. Mar. Sta., Bull., 2, 1945: 1–54, pls. 1–10, charts 1–2. $1.00.—Zoologists who may think that the time for faunal surveys by competent specialists is past would do well to peruse this treatise on the annelids of the Beaufort region. The number of species known from the region is about doubled. Two new genera and six new forms are described, and numerous other systematic refinements made. A variety of interesting ecological associations and habitat niches are confirmed or discovered, and are conveniently listed. Other natural history data, as characteristic macroscopic egg masses and characteristic trails or surface marks or tubes, are likewise epitomized. The annelid fauna of the Beaufort area is regarded as relatively rich, due to a mixture of boreal and tropical types. Such studies open the way for research in many biological fields.

SHORTER ARTICLES AND DISCUSSION

GEOGRAPHICAL REGULARITY IN THE VARIATION AND SUPPOSED MIMICRY OF A BUTTERFLY, *LIMENITIS BREDOWII*

THE geographical variation in the butterfly *Limenitis bredowii* has recently been studied by Carpenter and Hobby (1944). This is a North American species very closely related to the tropical American genus *Adelpha*. Forms of the latter are distributed generally in the American tropics and in some places extend slightly beyond the tropics of Cancer and Capricorn. *L. bredowii* occurs from Gutemala through the Mexican plateau to coastal Oregon, Utah and Colorado. Other species of *Limenitis* (or of *Basilarchia,* as the group is better known) are to be found over the whole of North America from the Mexican plateau to southern Canada. It is a matter of individual opinion which generic name should be used for *bredowii.* Sometimes a separate genus, *Heterochroa,* is adopted for this species alone.

Butterflies of these genera are characterized by a basic wing pattern usually consisting of a black ground color broken by white, bluish or orange-brown spots or bands. The relative position of these spots and bands is always the same in the various species; they differ, however, in the size, shape and intensity of color in the light areas.

Limenitis bredowii has been divided into four geographical subspecies inhabiting an equal number of north to south regions, namely, *californica* along the Pacific Coast north of Mexico, *eulalia* in northern Mexico and the adjacent mountain states in the United States, *bredowii* in southern Mexico north of the Isthmus of Tehuantepec and *guatemalensis* in the Guatemala highlands.

Carpenter and Hobby have obtained statistics on the varying size and color characteristics in an attempt at illustrating graphically the convergence of the pattern of this species toward that of the tropical species of *Adelpha* in the region of supposed contact with it in Guatemala. *L. bredowii guatemalensis* is more like some species of *Adelpha* than is the subspecies *californica,* which does not come in contact with *Adelpha.* The implication made by Poulton in 1908 and Carpenter and Hobby now is that of the existence of mimicry between these two groups in the zone of distributional overlap. It is only fair to state that the authors recognize that a convergence in pattern alone does not prove mimicry.

An explanation alternative to mimicry is that the convergence of *Limenitis bredowii* toward *Adelpha* is related to the physical factors of the environment; such parallel ecogenotypical color variations are shown by many animals. The convergence toward *Adelpha* then would be caused by the action of similar environmental agencies on all the butterflies and mimicry need not be postulated.

The geographical trends of variation in the color pattern may be summarized from the data of Carpenter and Hobby for thirteen characters (Table 1). Ten of these thirteen characters are related to a general lightening or darkening of the color of parts of the wings. That is, where the width of a white band is wider in one species than the others, that subspecies has a lighter appearance than the others. The index number (1) has been given to the one of the four subspecies which presents the darkest manifestation, the number (2) the next darkest, etc. Where two or more subspecies are not different in the manifestation of a character, they are given the same number. The four subspecies are arranged in order from north to south. By taking the sum of the numbers thus obtained for each subspecies or geographical area, it is possible to get an index of the degree of its darkness or lightness in color. From north to south, it is clear that such a definite relation does exist. Out of a possible minimum of ten points for darkness, the northern subspecies *californica* has 12.

TABLE 1

Character number used by Carpenter and Hobby or description	North			..........South
	californica	*eulalia*	*bredowii*	*guatemalensis*
Color of forewing band	1	2	2	2
4	2	2	1	3
5	1	2	3	3
7	1	2	3	3
8	1	2	3	2
9	1	2	1	3
10	2	1	3	3
12	1	2	2	3
13	1	2	2	2
14	1	2	2	2
Sum of ten characters	12	19	22	26
Shape of hind wing band	1	2	3	3
11	1	1	2	2
15	1	1	3	2

Out of a possible maximum of 27 points for lightness the southern subspecies *guatemalensis* has 26. The other subspecies enter into the gradient as expected.

The other three characters have no obvious connection with darkness or lightness. It is possible that they are physiologically linked with the climatic change in the same way, but the data do not justify general conclusions.

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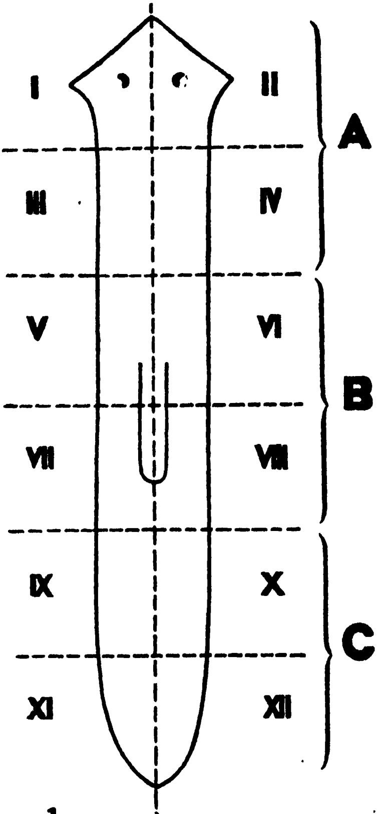
A NOTE ON LATERAL PIECE REGENERATION IN *PLANARIA GONOCEPHALA*

Recently, Rulon (1940) has given us a brief review of the subject of experimental control of regeneration in *Planaria* in which it is shown that the work of Child and his associates was concerned with control of correlative factors present in the regenerate, as well as control of regeneration, through the use of various external agents. The fact that a planarian fragment can, under favorable conditions, reorganize itself into a symmetrical and normally proportioned entire worm is well known, but in the course of reconstitution, the regenerating worms often exhibit a certain degree of temporary morphological asymmetry and disproportion. Despite the extensive literature on *Planaria* regeneration, the spatial and temporal relations of symmetry regulation and of the establishment of normal proportionality, and the factors controlling these processes have, so far as we know, been largely ignored. With a view to obtaining information on these points, a careful analysis of lateral piece regeneration in *Planaria gonocephala* is now being undertaken. Experiments already performed show that there is a difference in the rate of symmetry regulation for lateral pieces taken from different physiological levels; sometimes this difference is confined to difference in morphological characteristics. We have also noted what appears to be a regular spatial relationship between the mode of reconstitution and some invisible ground pattern of the sectioned mother worm. It is the purpose of this preliminary note to give an account of some of these observations.

EXPERIMENTAL DATA

A mature worm which had been starved for a week was cut into twelve pieces in the manner shown in Fig. 1. The pieces were numbered I, II, III to XII, respectively. They were cultured separately, but a group of ten corresponding pieces taken from ten different mother worms was placed in a single dish,



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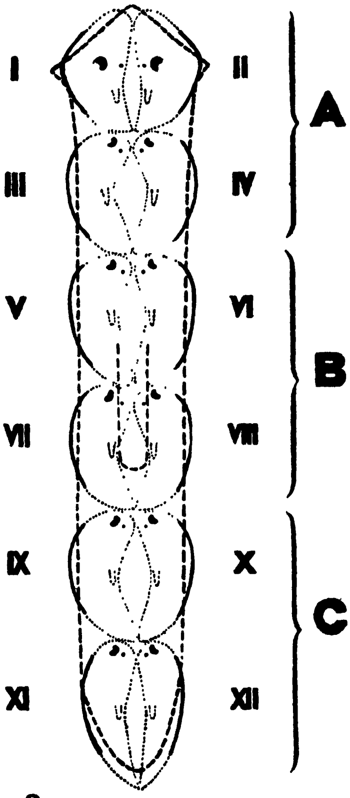

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FIG. 1. Diagram showing the manner of sectioning in obtaining different lateral pieces (I to XII) used in this investigation. Letters A, B and C denote the prepharyngeal, pharyngeal and postpharyngeal region, respectively.

FIG. 2. Diagram showing the general relationship between the reconstituted worms and the original animal. The asymmetric configuration with respect to body curvature, eye condition and pharynx position is discussed in the text. Notations have the same meaning as indicated in Fig. 1.

so as to keep them under identical external conditions, in order to make the results comparable.

The original body axis of each lateral piece could be easily recognized during reconstitution by the different pigmentation of dorsal and ventral surfaces and by the difference between the original intact lateral body wall and the newly healed one. Using these characters as landmarks, it was found that the antero-posterior axis of any reconstituted new worm coincides with that of the mother worm (Fig. 2). This agrees fairly well with the results of experiments made by Bever and Child (1930).

Three clear-cut characteristics: Body shape; number, size and location of eyes; and position of pharynx were chosen for the analysis of symmetry regulation, and changes in these during reconstitution were followed. Transient asymmetry with respect to these characteristics was common to all pieces, quite irrespective of the level of the mother worm from which they were derived. In a total of some 200 reconstituted worms examined during the early period of reconstitution, not a single one showed any symmetric character. At a later stage, however, each lateral piece regulates to form a symmetric and well-proportioned worm.

As regeneration proceeded, the antero-posterior body-axis of a regenerate elongated along a direction inclined always towards the median cut surface, so that in moving the fragment pursued a curving path. In a pair of lateral pieces from any given level, the peculiar body shape of one was the mirror image of that of the other (Fig. 2). The data collected suggest that the relative rate of symmetry regulation for body shape is more rapid than for other characteristics. Thus, at a certain stage of reconstitution, a regenerate may have normal body shape while its eye condition and pharynx position still remain asymmetrical. As regards the level difference, those pieces taken from the post-pharnygeal region of the mother worm appeared to be able to regulate their body shape rather more rapidly than prepharyngeal and pharyngeal fragments, although this difference was not marked.

The regulation of eye symmetry generally took place in two successive stages. Starting with a single eye, the worm formed a pair of eyes dissimilar in size; later these became equal and symmetrically distributed. Without exception, the first eye was the larger member of an asymmetric pair and was located away from the median cut surface, while the smaller one was adjacent to this surface. Such regular spatial relations in eye regulation may possibly be linked to some invisible structural differentiation of the ground pattern of the mother worm in the medio-lateral direction. When the paired lateral pieces from a given level were each furnished with an asymmetric pair of eyes, their dispositions in the two fragments were mirror images of each other (Fig. 2). As has already been stated, the regulation of eye symmetry proceeded more slowly than that of symmetry of body form. Postpharyngeal pieces were found to possess higher regulative ability for eye symmetry than prepharyngeal and pharyngeal pieces, the difference being more pronounced than that for body shape regulation. At a stage at which postpharyngeal pieces were furnished with a symmetric and well-proportioned eye pair, prepharyngeal and pharyngeal pieces had only a single eye or an asymmetric pair. This is perhaps the most interesting result of our experiments.

The newly reconstituted pharnyx always occupied a position adjacent to the median cut surface as shown in Fig. 2. It was observed that those pieces derived from pre- and postpharyngeal regions reorganized more rapidly into worms with the pharynx symmetrically placed than did pieces from the pharyngeal region. The result indicates that the pharynx of the mother worm may exert some influence on the regulation rate of pharynx symmetry of the pharyngeal pieces.

From these observations it may be concluded that every lateral piece tends to reorganize itself into a worm indistinguishable from the normal individual in every respect except that of size, and that the antero-posterior axis of such a reconstituted *Planaria* coincides with that of the sectioned worm. The fact, however, that the regulatory process in each lateral piece is always preceded by a temporary asymmetry of certain structural characteristics is of some interest. The position may be summarized as follows: First, inclination of body curvature, asymmetric location of the reconstituted pharynx and the position of the small-sized eye are constant features in all regenerates irrespective of their original physiological level. Secondly, the asymmetric configuration of one member of a pair of lateral pieces from a given level is always a mirror image of that of the other. Thirdly, there is a certain difference in the relative rates of regulation of symmetry both with respect to pieces derived from different levels and with respect to different mutual characteristics. Experimental analysis is being continued.

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ON THE INVERSE RELATION BETWEEN INHERENT AND ACQUIRED PROPERTIES OF ORGANISMS·

IN studying the differences between various races belonging to the same species or between similar species of the same genus, it can be recorded that the stronger are their inherent properties in some specific respect, the less they are capable of strengthening these properties under the effect of environmental factors. The existence of inverse relation between the inherent and acquired properties of organisms can be brought into relation with the general property of living systems known as compensation. Geoffroy St. Hilaire realized that with the increase of some organs the others decrease. Rensch[1] mentioned recently several such examples. In these cases the compensation is due to a redistribution of the limited amount of matter in a biological system. It is highly remarkable, therefore, that there exist compensatory relations between acquired and inherent properties in such biological systems as a species or a genus.

Fig. 1 shows the relation between the inherent resistance to salinity and that acquired in the process of acclimatization, in various races of infusoria *Paramecium caudatum.*[2] The acquired resistance is distinctly inversely proportionate to inherent resistance, (Acquired) $= 1 - \alpha$ (Inherent). The coefficient of inverse proportionality (α) in this case is equal to unity. This case can be considered as an example of the complete compensation: the deficiency of inherent resistance is compensated by the acquired resistance in such a way that the total of these two

[1] *Biol. Rev.*, 14: 180, 1939.

[2] G. F. Gause, *Quart. Rev. Biol.* (in press).

properties is maintained approximately the same within the limits of the species. But it is also often observed[3] that $\alpha > 1$ (in the rapid acclimatization of paramecia to increased salinity $\alpha = 3.6$ and to quinine solutions $\alpha = 2.2$). These cases can be considered

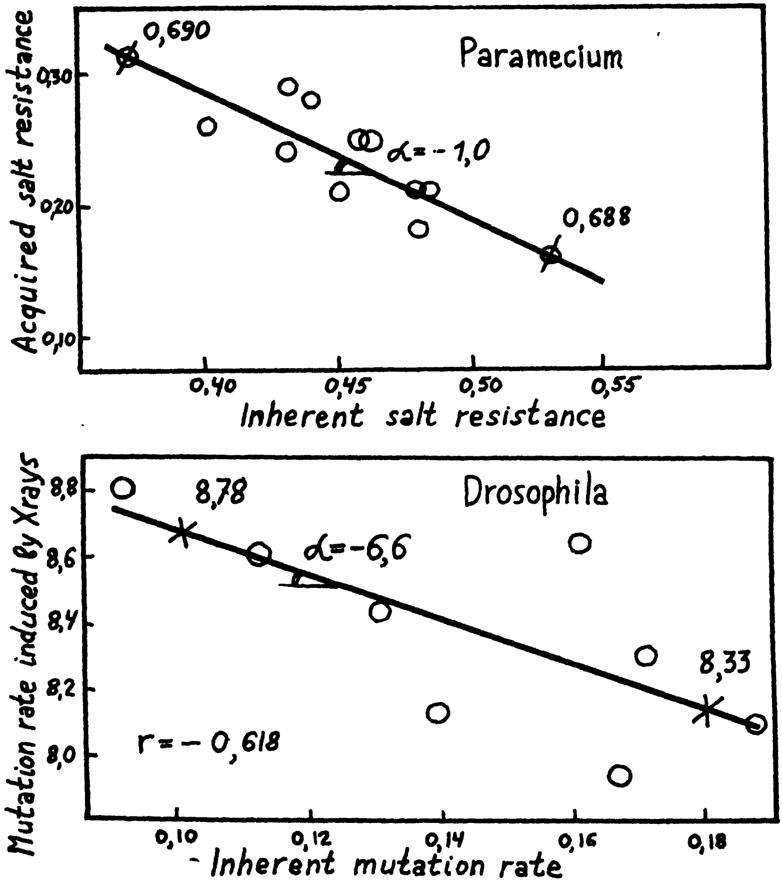

FIG. 1

as the incomplete inverse proportionality between inherent and acquired properties or, in other words, as a non-proportionate compensation. The inherently weaker races evidently overcompensate their weakness, and in respect of the total resistance they exceed inherently stronger ones.

The inverse relation between inherent and acquired resistance to arsenic in different races of infusoria *Colpoda steini* is clearly seen from the figures published by Schuckmann,[4] although the author himself did not notice this regularity.

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[3] G. F. Gause, *loc. cit.*

It is very interesting to refer to inverse relation between inherent mutation-rate and that induced by x-rays in various races of *Drosophila melanogaster.* Timofeeff-Ressovsky[5] concluded that these characters are not correlated with each other. When, however, we plotted his own figures on a graph (Fig. 1), the inverse relation between both variables was easily seen. The coefficient of correlation between the inherent and acquired mutation rates, $r = -0.618$, and having six degrees of freedom we can with the certainty of 90 chances out of 100 accept the existence of this inverse relation. The coefficient α calculated by the method of least squares equals to -6.6. As far as in the light of the data published by Demerec[6] the observations of Timofeeff-Ressovsky are not entirely comparable because of being made over a period of several years, it would be desirable if they were repeated by geneticists in the special experiments designed for that purpose. If confirmed, the process of mutation could be brought under the general principle of inverse relation between inherent and acquired properties of organisms.

The further example is the inverse relation between the inherent number of erythrocytes in blood of different species of mice belonging to the genus *Apodemus,* and the number of erythrocytes acquired under the effect of decreased barometric pressure.[7] In this system, as in those mentioned earlier, the acquired properties are inversely proportionate to inherent ones.

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[4] *Biol. Zbl.,* 60: 239, 1940.

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[6] *Genetics,* 22: 469, 1937.

[7] G. F. Gause, *loc. cit.*

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CLASSES AND TYPES OF INTRASPECIFIC INCOMPATIBILITIES[1]

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IN respect to the locus of sexual reproduction in the life cycle and to the scope, action and genetics of the modifying factors of incompatibilities, there are three main classes of incompatibilities which may be designated as follows:—

I. *The diplont-haplont class.* In the flowering plants the inhibiting effects of self- and cross-incompatibilities occur chiefly during reactions between the diploid tissues of the pistils of flowers of the sporophytic generation and the haploid pollen tubes of the alternating gametophytic generation.

II. *The haplont-haplont class.* In the fungi the reactions of incompatibilities occur in the interrelations between individuals that are haploid, bisexual and gametophytic.

III. *The diplont-diplont class.* In the ciliates among animals the reactions of incompatibilities operate chiefly in the relations of selective conjugation between individuals that are bisexual and diploid and hence comparable to the sporophytic generation in plants.

I. THE DIPLONT-HAPLONT INCOMPATIBILITIES IN FLOWERING PLANTS

Self-incompatibility. A self-incompatible individual must of necessity be bisexual (hermaphroditic). The identification and study of incompatibilities is most definite when there are no conditions, such as herkogamy and dichogamy, which limit or prevent the necessary association of elements of sexual reproduction. The flowering plants included in this discussion, unless otherwise stated, have perfect homomorphic flowers without any conditions or developments which limit or prevent self-, close- and cross-pollination.

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[1] Address delivered before the Torrey Botanical Club, May 16, 1945.

Self-incompatibilities ("self-sterility") were recognized some 150 years ago in the early studies of "flower pollination" and hybridization. Darwin and his contemporaries observed in a number of species that the pollen of stamens may be inactive on the stigma of the same flower. But Darwin (1876) did not recognize that there is also cross-incompatibility among plants that have homomorphic flowers. He stated in 1876 that "We may confidently assert that a self-sterile plant can be fertilized by the pollen of any one of a thousand or ten thousand individuals of the same species." The authority of this statement no doubt greatly delayed recognition of cross-incompatibilities.

Intraspecific cross-incompatibilities were first definitely recognized and related genetically to self-incompatibilities by Correns (1912). But his interpretation considered that the reactions are determined by diploid genetic constitutions.

The first satisfactory evaluation of both the reactions and the genetic determination of self- and cross-incompatibilities in homomorphic flowering plants was by Prell (1921). He recognized that the incompatibility reactions are between *diploid* tissues of the pistils and *haploid* pollen tubes. He postulated a single allelic series of multiple, *oppositional* genetic factors (O_1O_2; O_1O_3; O_1O_4; O_2O_3; &c.). The primary reaction was considered to be the independent self-antagonism of one factor (O_a or O_b) in a haploid pollen tube with the same factor in the diploid (O_aO_b) tissues of the pistil either in selfing or in crossing. In self-incompatibility and in complete cross-incompatibility the two haploid classes of pollen together possess both of the incompatibility factors present in the pistils. Thus the two factors in the diploid plant are allelic but merely oppositional in position. Prell considered (1) that the self-incompatible plants are normally heterozygous for the special factors; (2) that there is what may be called hidden incompatibility and elimination of the genotype of the seed parent in the progeny when two parents possess one factor in common; (3) that there is elimination of the genotypes of both parents in a progeny when there is no common factor; and (4) that there may be induced or anaphylactic reactions due to cytoplasmic influence.

Applications of Prell's hypotheses were first made in experimental studies of incompatibilities in flowering plants of which reports by several persons were published in 1925, 1926 and 1927. The data demonstrated various critical features of behavior postulated by him.

1. THE PERSONATE MODE OF INCOMPATIBILITIES

The term "personate" was applied by Correns (1928) to what is the simplest mode of diplont-haplont intraspecific incompatibilities in homomorphic bisexual flowering plants. All the important and critical features of this mode have now been demonstrated and may briefly be summarized as follows:—

A. *In regard to mating reactions:*—1. The individuals are self-incompatible (S). 2. There are at least *three,* and often more, mating groups in the population of a species. 3. The members of each group are cross-incompatible. 4. As a rule the members of any two of the mating groups are cross-fertile.

B. *In regard to genetic relations and reproduction:*—1. Both self- and cross-incompatibilities are determined by the same genetic mechanism. The simplest personate mode in flowering plants involves at least three factors (usually designated S_1, S_2 and S_3). 2. The progeny of two plants comprise either two or four mating groups. 3. When there are two groups in a progeny there is hidden incompatibility which gives the elimination of the genotype of the seed parent and the appearances of a third genotype, as I $(S_1S_2) \times$ II $(S_1S_3) =$ II (S_1S_3) and III (S_2S_3). This is due to the self-incompatibility reaction of the common factor (S_1). 4. When there are four mating groups in a progeny there is elimination of both parent genotypes:—I $(S_1S_2) \times$ IV $(S_3S_4) =$ II (S_1S_3); III (S_2S_3); V (S_1S_4); and VI (S_2S_4). 5. The simplest or minimum sustaining population is one with three S factors and three mating types (S_1S_2, S_1S_3, S_2S_3). The progenies of the six reciprocal matings will, under natural free cross-pollination, provide interpolations that result in a balance of genotypes. 6. A population in which there are four S factors will consist of six mating groups, there will be thirty reciprocal cross-matings, and the reciprocations will tend to balance the different mating genotypes. 7. The incompatibilities effect selective reproduction *within* the populations of a single species. They are entirely distinct from interspecific hybridizations. They are intraspecific. 8. The mating is *bi-genotypic.* Self- and intra-genotypic reproductions are eliminated, and homozygotes for S factors do not appear normally.

Adequacy of the genetical analyses. In various species, or in such segments of them as have been studied, there are definite and effective expressions of the incompatibilities by a single allelic series of S factors. It is, however, to be recognized that the quality and strength of the various S factors differ not only among different species but in a single species. Such differences in expression as (a) end-season fertility, (b) cyclic incompatibility, (c) differences in the observed behavior of pollen tubes, (d) partial or feeble fertility, and (e) border-line action of certain factors have been recognized.

The fundamental nature of incompatibilities in relation to the fertility of sexual reproduction. It is recognized that in the personate mode the S factors comprise an allelic pair in each of the diploid individuals that mate.

Since plants which mate are different in one or in both *S* factors it is often considered that these factors effect fertility when in this condition. Also in various hybridizing relations between species, an *S* factor of the one is often represented as allelic to a fertility factor of the other. I am convinced that this is not an adequate and correct evaluation. As early as 1931 it was suggested (Stout, 1931) that the special factors of incompatibilities are superimposed on the fundamental mechanism of intraspecific fertility in a species. This view has again been stated in connection with a report that incompatibility factors may be inactivated when there is a change to tetraploidy (Stout, 1945).

A further consideration of this matter may now be made as follows:—1. There are no special sexual organs or elements exclusively involved in the reactions of incompatibilities. They operate by inhibiting an action that is already established as a necessary step in sexual reproduction. 2. Incompatibilities exist in certain species of various families of flowering plants; but, of several closely related species that readily hybridize and are very similar, some may have incompatibilities and others may have none. This emphasizes the fact that no aspect of these incompatibilities is an obligate concomitant of sexual reproduction. 3. There is general agreement that *S* factors are *inactive* in effecting incompatibility unless there is self-interaction. 4. There is evidence that inactivations of *S* factors are increased in polyploid flowering plants; and that there may be complete inactivation which is transmitted to seed progenies. 5. Incompatibility reactions may be reduced or avoided by special means such as removing secretion from stigmas or by premature pollination. In cyclic incompatibility there is fertility in the mid-season of flowering. When incompatibility factors do not operate effectively there is fertility of interspecific sexual reproduction.

It is concluded that incompatibilities are physiological and inhibiting reactions that are superimposed on, and secondary to, the more fundamental mechanisms and features of sexual reproduction which will be referred to as *residual fertility.* We may consider that special genetic factors effect epistatic incompatibility reactions and that when these are inactive there is the action of intraspecific fertility for which every homomorphic bisexual individual is fully equipped.

2. THE ASSOCIATE MODE OF INTRASPECIFIC INCOMPATIBILITIES

The interpretation of what is called the associate mode of incompatibilities was postulated by Kakazaki in 1930 to explain the complicated reactions which he observed in cabbage (*Brassica oleracea*). His conceptions have been rather fully evaluated, tested, and applied in connection with the behavior of incompatibilities in a diploid variety of garden petunias known as Rosy Morn (Stout, 1938). The recognition of the features and genetics of this mode aids greatly in the evaluation of incompatibilities that do not conform fully to the personate mode.

The features which characterize this mode of incompatibilities, in comparison with those of the personate type, may be summarized as follows:—

(A) *In respect to reactions of mating types:*—1. The population consists of self-incompatible (*S*) and self-fertile (*F*) individuals and mating groups. 2. There are numerous *S* and *F* genotypes in the entire population. 3. There are both cross-fertility and cross-incompatibility between *F* genotypes. 4. There are reciprocal differences in reactions. 5. The members of a group which react uniformly with various others may, when more widely tested, prove to be composed of two or more minor groups. Thus a mating group may be polygenotypic. 6. The determination of the ultimate mating groups often requires numerous collateral and reciprocal testings.

(B) *In respect to progenies of the various matings and their reaction groups:*—1. The number of mating types in different mating progenies range from one to as many as 12. 2. The selfed progenies of *F* plants segregate into both *F* and *S* genotypes. *F* plants do not breed true. They carry factors that are inactive or that exhibit hidden incompatibility in both selfing and in cross-fertility. 3. The progenies of reciprocal crosses differ in the number and the character of the mating groups. 4. The eliminations in a progeny effected by hidden incompatibility may be as many as six genotypes. 5. The eliminations complicate genetical analysis and effect complicated alternation of parent genotypes in a succession of line progenies. 6. The interpolations of genotypes of the various progenies are complex in respect to balance in populations.

(C) *Evaluations of reactions and genetic determinations:*—1. The evaluation that best accounts for all the reactions and progenies considers that there is one allelic series of three S factors (S_1, S_2, and S_3) and one allelic series of two special oppositional factors called T_1 and T_2, which favor fertility. 2. The reactions in haploid-diploid relations are $SSTT \times ST$. There is independent self-interaction for each factor. T factors interact to favor pollen-tube growth when they are alike in both pistil and pollen; S factors are inhibiting when alike; otherwise there is inactivation. But there are one S and one F factor in each pollen grain and their reactions are often in conflict; and there are neutral and epistatic relations in regard to the growth of the pollen tube. 3. This interpretation recognizes that not only incompatibility factors but also special factors which favor fertility may be superimposed on fundamental intraspecific fertility. 4. Except for the selfing and intra-crossing among each of the F genotypes, which do not breed true, all reproduction is bi-genotypic. For further discussion and experimental data and for charts showing patterns of population matings, hidden incompatibility and numerous patterns for progenies, reference may be made to a previous publication (Stout, 1938).

3. MORE COMPLEX MODES OF INCOMPATIBILITIES IN TETRAPLOIDS

A brief mention may be made of the more complex modes of incompatibilities that have been recognized. It is significant that these have been found especially, if not chiefly, in natural species and cultivated plants that are considered to be polyploid. In some cases at least these are of such long existence that the processes of meiosis have become standardized on a bivalent basis. Some of these amphidiploid species have evidently arisen from diploids that had personate incompatibility while others arose from diploids which had an associate mode.

The pollen of such tetraploids contains at least two *S* factors instead of one, and in many cases these are different. But a pollen tube is a single unit of growth and reaction and it is not able to exhibit two different reactions at the same time. Its two or more *S* factors, and also any special fertility factors that are present, may supplement each other in the same effect, compete in different reactions, exhibit a complementary effect, and produce new inhibitions or inactivations. There are the combined reactions in pollen grains, in tissues of pistils, and in the relations between the two. Reactions between 4n pistils and 2n pollen tubes provide opportunities for complex qualitative and quantitative interrelations. Every aspect of incompatibilities and fertilities seen in the diploids are present in tetraploids in various degrees of increased complexity.

Amphipersonate incompatibilities. Several earlier studies of *Verbascum phoenecium* indicate that the pattern reactions of progenies are not characteristic of the personate mode. More recently it has been considered that the members of this species are allotetraploid, that they possess two different allelic series of *S* factors (S_aS_b Z_aZ_b, &c.) and that there are multiple factors in each series in all possible homozygous and heterozygous combinations. When two *S* plants, that are heterozygous for both pairs of *S* factors and have no *S* factor in common, are cross-bred the progeny can comprise as many as 16 genotypes. When a progeny of no more than 20 plants are obtained and tested certain mating groups in their pattern may be polygenotypic. It appears that

Verbascum phoenecium may be called amphipersonate in its incompatibilities.

Amphiassociate incompatibilities. Cardemine pratensis may be considered as a species which has amphidiploid associate incompatibilities. From the results of studies with this species, Correns in 1912 first definitely recognized intraspecific cross-incompatibilities in homomorphic flowering plants and suggested a genetic status. But this was based on conceptions of dominance and recessiveness of Mendelian factors and the "sterility" was considered to be a diploid character. The diplont-haplont relation was not then conceived and Correns considered that the plants which he studied could be placed in only four mating groups.

Beatus (1934) has more recently studied this species and his reports indicate a tetraploid associate mode of incompatibilities which involves multiple factors in each of two allelic series of S factors and also in each of two allelic series of F factors. He postulates all combinations of heterozygocity and homozygocity in the various individuals for each factor of the four series. On this basis as many as 16 genotypic classes of pollen may segregate in the pollen of a plant or in the egg cells in ovules and as many as 256 genotypes are possible in the offspring of two parents.

Evaluations. You may feel that such complexities tax one's imagination and prohibit complete experimental demonstration. They do, but various of the most essential features of such complexity can be and have been determined. In comparison with the more simple personate and associate modes these polyploid cases exhibit greater complexity in all the features of incompatibilities and fertility such as:—the number of mating groups; border line and indefinite reactions; hidden incompatibilities; indirect heredity; elimination and rotation of genotypes in line bred progenies; increase in inter-genotypic incompatibility; and increase in polygenotypic major mating groups.

It is obvious that polyploidy increases the number of allelic series and multiplies the number of factors in tissues of pistils and in pollen grains. This compels multiple complementary reactions instead of the single-factor reaction in a pollen tube of a personate diploid.

Increased fertility. One feature of behavior in the incompatibilities of polyploids merits special mention. Repeatedly the amount of cross-fertility exceeds expectations that are based on the effective independent self-interaction of each of the various S factors that are present. Various investigators have considered that a polyallelic relation may be fertile if there is a *difference in only one* of the incompatibility factors. For example an amphipersonate relation of $S_1S_2Z_1Z_2 \times S_1S_2Z_1Z_3$ may be fertile on account of the functioning of S_1Z_3 and S_2Z_3 pollen. Thus it may be considered that the inaction of one factor (S_3) in the pollen may be accompanied by the inactivation of another S factor which is common in both pistil and pollen (S_1 or S_2). Such inactivations automatically give residual fertility the opportunity to function.

4. INACTIVATION OF INCOMPATIBILITY FACTORS IN POLYPLOIDS

The complete inactivation of all incompatibility factors has recently been demonstrated in autotetraploids of *Petunia axillaris* that were obtained experimentally from known personate diploids (Stout and Chandler, 1941; *idem,* 1942; Stout, 1945). Tetraploid branches were obtained on each of 15 different diploid plants by treatment with colchicine. The self-pollinated flowers of all these branches produced fine capsules well filled with viable seeds, while the flowers of the diploid branches on the same plants remained self-incompatible. This inactivation of incompatibility factors was inherited in all seedlings grown from the selfed seed of tetraploid branches, and also in all of over 300 seedlings of two later generations. Also several thousand of the cross-relations between tetraploid plants (intra-series, inter-series, 4n parents and 4n progeny, etc.) were tested by controlled pollinations. In every one of these intra-tetraploid cross-relations as well as in the selfed-relations many viable seeds were produced. The change from incompatibility to complete residual fertility occurred in the somatic tetraploidy of known personate diploids in which the two S factors were doubled ($S_aS_aS_bS_b$) and it continued in the *selfed* progeny.

In Trifolium repens. In similar studies with *Trifolium repens* recently reported by Atwood (1944) self-fertility first appeared in the seedling progenies derived by crossing two tetraploids which possessed no common S factor (the diploids from which they were obtained were (S_1S_2 and S_3S_4). Of the 29 seedlings of the first 4n progeny ($S_1S_1S_2S_2 \times S_3S_3S_4S_4$) three were self-incompatible and 26 were self-fertile and also cross-fertile. These results indicate that some of the recombinations (assumed to comprise either three or four S factors; as $S_1S_1S_3S_4$ or $S_1S_2S_3S_4$) effected self-fertility while others (assumed to involve two S factors as $S_1S_1S_2S_2$) continued with self-incompatibility.

Retention of self-incompatibility in tetraploids. But there are other reports which indicate that self-incompatibilities and also cross-incompatibilities may continue in tetraploids experimentally derived from self-incompatible diploids. Howard in 1942 reported that all tetraploid branches derived from *Brassica Rapa, B. campestris* and *Raphanus sativus* which he studied were self-incompatible. Hecht (1943) reports that tetraploids of *Oenothera rhombipetala* grown from colchicine treated seeds obtained from S plants were all self-incompatible, that the seedlings obtained from crosses of two such plants were self-incompatible, and that cross-incompatibilities and cross-fertilities continued in the seed-grown progenies.

Thus the tetraploids of some species exhibit complete inactivation of S factors while others do not. The basis for this is, no doubt, that there are differences in the quality and strength of the heredity factors themselves. Experimental studies of known tetraploids in comparison with their known parents will no doubt provide data for more adequate evaluations of the principles involved in the fundamental properties of *S* factors, especially in regard to inactivations.

5. CYTOPLASMIC DETERMINATION OF POLLEN-TUBE REACTIONS IN CAPSELLA GRANDIFLORA

The early studies of incompatibilities in this species indicate that there are few mating groups but a rather complex genetic behavior in the segregations in progenies. In 1936 Riley (1936) reported that there are three intra-incompatible but cross-fertile groups, which agrees with a minimum sustaining population of the personate mode. But the patterns of mating groups in the progenies were quite different. The results indicated that one group ("A") is poly-genotypic and composed of three genotypes (*Tt*ss; *Tts*s; and *Ttss*); that group "C" is bi-genotypic (*tt*ss; *tts*s); and that group "B" is mono-genotypic (*ttss*). This evaluation considers that there are two allelic series each having two incompatibility factors, that the four factors have successive relative epistatic powers both within a series and in complementary reactions, and that all the pollen of any individual plant has the same reaction due to cytoplasmic transmission of the diploid character of that individual. This effects a diploid-diploid relation.

The recombinations in the progenies of all fertile matings give a population of six genotypes which however exhibit only three mating groups.

The features of epistatic relations in complementary reactions may prove to be rather frequent not only in diplont-diplont relations but also in haplont-haplont relations. The feature of cytoplasmic determination of pollen-tube reactions may be frequent in the incompatibilities of flowering plants.

II. HAPLONT-HAPLONT INTRASPECIFIC INCOMPATIBILITIES IN FUNGI

In the fungi the locus of both vegetative activity and sexual reproduction is in the haplophase of the life cycle.

This automatically reduces the genetic determination and expression of incompatibility reactions, as of other segregating characters, in each haplont to only one factor of any allelic series or to complementary reactions between one such factor of each of two or more diploid allelic series.

Mating types which involve incompatibilities and effect selective reproduction in fungi are well exemplified and most extensively known in various species of Hymenomycetes, chiefly through the experimental studies made by Bensaude, Vandendries, Brunswick, Hanna and Kniep. Brunswick in 1924 recognized that the failures in mating reactions involve self-sterilities similar to the oppositional reactions postulated by Prell. But Kniep (1928) and more recently Buller (1941) have applied the doctrine of multiple sexes and unisexual heterothallism.

1. INCOMPATIBILITIES IN HYMENOMYCETES

Bipolar incompatibilities with multiple mating groups. In the simplest mode of haplont-haplont incompatibilities, as in *Coprinus comatus,* a sustaining population comprises two mating groups, but the entire population may comprise several such bipolar groups (S_1 and S_2; S_1 and S_3; S_2 and S_3; S_4 and S_5; &c). These may be isolated as geographical races. The members of each mating group do not copulate, and for them there is cross-incompatibility, as S_1 with S_1. But when members of any two mating groups have the opportunity to copulate there are fusions of mycelia which effect reciprocal cross-fertilization except when two races are alike in one (S_1 and S_2 with S_1 and S_3) or in both of the factors.

When the sustaining population of a species comprises only two mating types the mating appears superficially like unisexual heterothallism that is due to genetic segregation of physiological maleness and femaleness or of realizator factors in meiosis. But when there are multiple mating types one must fully evaluate the condition of bisexual heterothallism that involves the action of multiple factors which effect intraspecific incompatibilities.

When the genetic "sex factors" assumed by Kniep are assigned as incompatibility factors the reactions fall into perfect accord with those of the personate incompatibilities in the diplont-haplont relations with the single difference that the mycelia of the fungi are haplonts.

Tetrapolar incompatibilities. Kniep's data for the fungus *Schizophyllium commune* reveal that a sustaining population may comprise two pairs of cross-fertile mating groups but the members of each pair are cross-incompatible with both of the other pair. Also the genetical analysis revealed that the progeny of any cross-fertility segregates into all four mating groups.

Kniep's evaluation considers that there are two allelic diploid series of sex factors which give haploid segregations of two females (*AB* and *Ab*) and two males (*ab* and *aB*); that *AB* × *ab* and *Ab* × *aB* are inter-sexually fertile; that *AB* × male *aB* is sterile. The diploid of any fertile relation is *AaBb*.

Treated as incompatibilities between bisexual haplonts the genotypic diploid constitution is $S_1S_2Z_1Z_2$ and the haplont genotypes are S_1Z_1; S_1Z_2; S_2Z_1; S_2Z_2. In two combinations ($S_1Z_1 \times S_2Z_2$ and S_1Z_2 and S_2Z_1) there are no self-interactions and residual fertility operates. In the combinations $S_1Z_1 \times S_1Z_2$ and S_2Z_1 and also $S_2Z_2 \times S_1Z_2$ and S_2Z_1 there is incompatibility effected by one common factor. The incompatibility factors segregate in meiosis to give four kinds of haploid hermaphrodites; there is only *one diploid genotype and it is heterozygous* for both allelic pairs of incompatibility factors.

Multiple races with tetrapolar incompatibilities. These exhibit the extension of incompatibilities in a species by the genetic action of multiple alleles in one or in both of *two allelic series* of *S* factors. In the species *Coprinus lagopus* (= *C. fimetarius*) Brunswick determined 729 genetically different haplonts and soon thereafter 24 different tetrapolar races of this species were recognized by Hanna (1925). Within each race the patterns of the reactions and of the genetic segregations are as in

Schizophyllium commune. But when these different races are interbred all inter-relations are fertile. On Kniep's and Buller's assumptions there are many kinds of males and many kinds of females and in the inter-relations between races any female group is cross-fertile not only with all males but with all other females; and any male group is cross-fertile with the other males of inter-race relations.

Evaluations. Kniep continually interpreted the failures in mating of the mycelia in these fungi as a reaction between two sexes. Species whose monosporous mycelia did not fructify unless mated were classed as dioecious in contrast to those that were self-reproducing and bisexual (homothallic). Multiple mating is, in this view, merely an extension of the mating of unisexual males and females and the non-mating of any two of the same sex. It was assumed that there are numerous quantitatively different males and females which are differentiated by quantitative values of the numerous sex factors that determine both the successes and the failures of copulation.

Brunswick in 1924 rejected this view of Kniep's and considered that the failures in copulation involve *sterility* factors with the action of oppositional factors as postulated by Prell. He noted that the phenomena of mating in these fungi are very similar to the so-called self-sterilities in hermaphrodite flowering plants. It seems rather obvious that this is the correct view.

In evaluating these reactions of mating the first consideration is whether or not a mycelium is functioning as a unisexual or as a bisexual individual. An individual haploid mycelium of the particular species of fungi under consideration must be, it seems to me, recognized as bisexual for the following reasons. The matings are copulations of apparently undifferentiated cells of two mycelia. There are no specialized branches that can be regarded as sex organs. The mycelia intermingle; there are multiple copulations; two cells that copulate come into close contact and an opening is formed through their walls. The nucleus in each conjugant divides by mitosis and the two sister nuclei become physiologically differentiated in the *first stage of nuclear sexuality.* The one that remains in its cytoplasm may be called female; the other, or male nucleus, migrates into the other conjugant cell. Thus there is an exchange of nuclei. Each conjugant fertilizes or spermatizes the other in the sense that a dikaryotic association of two semi-sexually differentiated nuclei is effected. Such behavior certainly indicates that each haploid mycelium is hermaphroditic at the time of copulation and before there is exchange of nuclei. The reactions of incompatibility are to be regarded as properties of the entire haploid body of each mycelium in their inter-relations. These properties are determined by genetic factors which segregate in meiosis. There may be further fusions of mycelia after dikaryotic association.

Nuclear sexuality, whether of male or female potency or of "realizator" factors, in these particular haploid bisexual and homomorphic fungi is not genetically segregated in meiosis but is a matter of somatic differentiation, as this and many other features of sexuality always are in any hermaphrodite either haploid or diploid.

It seems that many students of the fungi, especially Kniep and Buller, have very generally considered that any species of fungus whose members exhibit mating is haplodioecious and "heterothallic" and that the members are unisexual. This view fails to distinguish between true unisexual heterothallism and bisexual heterothallism which is effected by either dichogamous developments or by incompatibilities.

Further complexities have been noted in the Hymenomycetes, and in other fungi, in both the mating patterns of populations and of progenies; of these mention may be made of the following:—(1) Aversion or barrage reactions (Vandendries and Brodie, 1923; Biggs, 1938) in which one pair of allelic factors in a tetrapolar mating effect "aversions"; and (2) false clamp connections were also recognized by the authors just noted. But such behavior occurs after fusion. (3) Secondary fusions of mycelia and secondary exchanges of nuclei between dikaryonts and non-dikaryonts which effect the so-called legitimate and illegitimate exchanges of nuclei. (4) Epistatic complementary determination in the completion of factors in haplonts. (5) Presence of groups that are neutral. (6) Presence of apomictic life cycles. (7) Operation of both bipolar and tetrapolar incompatibilities in an inter-breeding population with "Durchbrechungskopulationen."

2. BIPOLAR INCOMPATIBILITIES IN THE RUST FUNGI

Incompatibilities in the rust fungi are well exemplified in the well-known barberry-grain rust, *Puccinia graminis.* Our consideration to-day involves only the evaluation of the barberry phase of the life cycle in which the haploid uninucleated bisexual mycelium accomplishes the fusions which effect the dikaryotic association of male and female nuclei of the dikaryophase of the life cycle.

The individual monosporous mycelium of an infection area in the barberry leaf is haploid and bisexual. The pycnia are somatically differentiated as aggregates of two kinds of branches; (1) those that produce pycnospores which function as male spores and (2) those that function as trichogynes in the process of effecting the dikaryotic association of potential male and female nuclei. There is hence a somatic differentiation of two haploid structures of importance in sexual reproduction and which may be compared, in relative significance at least, to the differentiations of diploid pistils and stamens in a flower or in a composite cluster of flowers.

Cragie (1927; 1931) demonstrated experimentally that in *Puccinia helianthii* and *P. graminis* the pycnospores or spermatia of a mycelium do not function in self-relations and that in respect to the cross-relations there are two intra-cross-incompatible but inter-cross-fertile mating groups. The mechanism of effecting cross-spermatization is surprisingly like that which effects cross-pollination in flowering plants which have perfect flowers. Secretion forms in droplets on the pycnia; flies feed on this secretion and transport the functionally male pycnospores from one individual haplont to others. The experimental tests revealed that the self-relations and cross-relations between members of a mating group fail to effect formation of aecia, but that cross-relations between members of two mating types are fertile.

It appears that only two mating types have thus far been identified in the tests that have been made in those rust fungi that exhibit *bisexual heterothallism.* This indicates a haploid genetic control by only one allelic pair of factors, in which case the constitution of the diploid nucleus in the life cycle of all individuals consists of *only one genotype.* This condition cannot exist in diploid flowering plants or in animals that have incompatibilities.

Bisexual heterothallism versus unisexual heterothallism. As recently as 1941, Buller (1941) listed *Puccinia graminis* (and also *Coprinus lagopus*) as heterothallic and as having "the sex separated one from the other in different haploid mycelia." According to this view the mating would be unisexual heterothallism. Buller failed to consider that the haplophase of *Puccina graminis* is actually bisexual and that incompatibilities which are superimposed on sexual differentiations effect self- and intra-mating group cross-incompatibilities. That this is the condition has been recognized by Jackson (1944) who stated that the mycelia of *Puccinia graminis* and of certain other bisexual heterothallic fungi are not "thalli of opposite sex" but are actually "bisexual (hermaphroditic) self-sterile, and inter-fertile." It may be emphasized that there are also cross-incompatibilities among the members of each mating group.

3. BIPOLAR INCOMPATIBILITIES IN ASCOMYCETES

Recent studies, summarized by Jackson (1944), have demonstrated "the widespread occurrence of a spermatial (microconidial) type of sexuality in the Ascomycetes.'' The information also definitely indicates widespread bisexuality and bipolar incompatibilities among many cases of so-called ''heterothallism.'' In such cases there is segregation of the *S* factors in meiosis and for the sexuality of nuclei there are somatic differentiations. The expressions of sexuality, especially of nuclei, are masked by (a) the multinucleated condition of both haplont and dikaryont mycelia, (b) by the loose dikaryotic association of nuclei during a period of extended vegetative activity, (c) by diversities in the extent of nuclear migrations, and (d) by the reversibility of nuclear sexuality.

In *Sclerotinia Gladioli* Drayton (1934) reported that a mycelium is a bisexual haplont which produces both spermatia and receptive trichogynes. But there is bipolar mating which involves self-incompatibilities, intramating group cross-incompatibilities and inter-group fertility. In this obligate parasite there is a short life cycle with the dikaryotic association limited to a few generations of ascogenous hyphae.

Hermaphroditism in dikaryotic ascospores. In certain species such as *Pleurage anserina* (Ames, 1932, 1934) and *Neurospora tetrasperma* (Dodge, 1928, 1936, 1942) the mechanism of ascospore formation provides each of the four ascospores in an ascus with two nuclei. Since a mycelium from such a spore is sexually self-reproducing it has generally been considered that these two nuclei are of opposite sex, male and female, and that it is their nuclear offspring that finally fuse. That the sex differentiation of these nuclei is not a definite and irreversible segregation has been shown by the following method. A nucleus may be isolated either in uninucleated ascospores or in uninucleated conidia. When a mycelium is grown from such a spore it will produce both spermatia and trichogyne-like structures and hence is a functional hermaphrodite. But such an individual mycelium is self-incompatible and a number of them exhibit bipolar mating incompatibilities. Thus the nuclear sexuality in the binucleated ascospore is reversible but the incompatibility factor remains constant. The incompatible reactions of the mycelium in mating should not be confused with nuclear reactions and with the nuclear sexuality of dikaryotic association, of pairing in ascogonous hyphae and finally of their fusion in the ascus. The two are quite different aspects of sexual reproduction. The binucleated dikaryotic ascospore is actually a double hermaphrodite with nuclear sex differentiation a matter of differentiation. Dodge has repeatedly called the mycelia that grow from these uninucleated spores "unisexual" solely because they do not produce perithecia unless mated. Ames very definitely recognizes that the uninucleated mycelia are hermaphrodites and that the failures in mating involve "self-sterility" factors.

Bipolar incompatibilities in Neurospora sitophila and in related species that have uninucleated ascospores have repeatedly been referred to by Dodge and by Buller as the mating of mycelia of opposite sexes. But Dodge (1939) states that "every mycelium of *Neurospora sitophila* develops (1) incipient ascocarps with receptive organs, (2) small microconidia and spermatia, and (3) large monilioid conidia." Hence each monosporous mycelium is a functional hermaphrodite. But there is self-incompatibility and also intra-genotypic cross-incompatibility. In the inter-genotypic mating there are fusions of mycelia and also spermatial and conidial cross-fertilizations. The incompatibility factors segregate in meiosis and effect bisexual heterothallism, but nuclear sexuality is a matter of somatic differentiation.

In *Hypomyces solani cucurbitae.* Hansen and Snyder (1943) have reported the operation of incompatibility factors in the matings between hermaphroditic mycelia and unisexual male mycelia that have evidently been derived from hermaphrodites by mutation. The self-incompatible bisexual mycelia comprise two mating groups that are intra-incompatible but inter-fertile (S_1 and S_2). But the male mycelia also comprise two mating groups. When a bisexual S_1 is mated with a male S_1, or a bisexual S_2 is mated with a male S_2 there is incompatibility. When a bisexual S_1 is mated with a male S_2, or a bisexual S_2 is mated with a male S_1 only the bisexuals produce perithecia. In these tests the hermaphroditic mycelia "are grown to maturity and have receptive perithecial primordia," and then the transfer of "any part of the living thallus, ascospores, conidia, or bits of mycelium can act as the male fertilizing agent" provided there is an inter-group relationship. The inability of the male mycelia to form perithecial primordia and to receive nuclei from hermaphrodites are, it would seem, adequate criteria of unisexual maleness. Hansen and Snyder note that sex and incompatibility are distinct and independent characters.

Concluding remarks regarding the fungi. In the fungi more critical studies and evaluations are needed to distinguish (1) the species whose members are able to function only as one sex, (2) the species whose members are bisexual and able to function in self-reproduction or in unrestricted intraspecific relations, and (3) those that are bisexual but whose intraspecific reproduction is limited by incompatibilities. Much further data are needed before an adequate evaluation can be made of the scope and role of the processes of cross-reproduction between bisexual mycelia which possess no incompatibilities. Homothallic reproduction may indeed be less obligatory than is usually assumed. It would seem that self-reproduction and unlimited intraspecific reproduction between sexes and sex elements must be a fundamental feature that precedes in evolutionary development the superimposed limitations of incompatibilities.

III. Diplont-Diplont Intraspecific Incompatibilities in Ciliate Protozoa

The discovery of selective reproduction and mating types in the diploid hermaphroditic species *Paramecium aurelia* was reported by Sonneborn (1937). For many years various students of this genus believed that any one individual of a species could mate with any other one, exchange nuclei, separate, and then continue in vegetative vigor and fission multiplication until the next mating period. This was frequently called "sexless reproduction," chiefly because there were often no morphological differences in the conjugants, but also because the two individuals are hermaphrodites. Zoologists have frequently insisted that there is no sex in hermaphrodites; meaning that in them there is no unisexualism such as is general in animals.

Investigations of other species and genera of ciliates soon followed (Jennings, Sonneborn, Kimball, and others) which revealed considerable diversity in the number and the behavior of the mating groups. But it appears that intraspecific incompatibilities, comparable in the most important features to those already discussed, are frequent if not the rule in the genus *Paramecium* and in related genera.

The most important of the nuclear processes in sexual reproduction in these unicellular animals are believed to be chiefly as follows:—Each conjugant is a complete animal which has at least one generative nucleus that is diploid at the time of mating. Hence the mating is a diplont-diplont reaction.

After conjugation begins there is meiosis in which only one haploid nucleus survives. This undergoes *mitotic division* and the two sister nuclei become differentiated in sex-behavior though identical in constitution. One nucleus remains in its cytoplasm; one migrates into the cytoplasmic body of the other conjugant. This formation of two differentiated nuclei is the first expression of nuclear sexuality. Then there is the mutual exchange of what we may call male gametes. This effects reciprocal cross-fertilization between members of two mating groups within each of which there is cross-incompatibility.

Certain features of this conjugation are similar to those in various of the fungi. In the fungi there is conjugation between cells of the haploid bisexual mycelia. In both there is somatic sexual differentiation of two sister nuclei with exchange and mutual cross-fertilization. In both the incompatibilities inhibit certain conjugations. In the fungi the reactions are haploid-haploid; in the ciliates they are diploid-diploid.

Bipolar incompatibilities in P. aurelia. Sonneborn (1927) found that the population of each of four different intersterile varieties of *P. aurelia* comprise only two mating types. At first these were called for convenience ''sex I and sex II'' but soon the designation was changed to ''mating types'' and ''caryonides'' for it was definitely recognized that all the individuals ''are functionally hermaphroditic, each conjugant of a pair fertilizing the other one'' (Sonneborn, 1938). Thus it was recognized that evaluations in terms of reactions between *two sexes* are inadequate. It is to be noted that in this case the different races of the one species exhibit the specificity barrier to hybridization equal to that between species that will not hybridize.

Multiple incompatibilities. More complicated mating systems were reported in *Paramecium busaria* by Jennings (1938 and 1939) and numerous researches by him have been reported since that date. Three varieties were identified; these were intersterile, due it appears to varietal specificity and not to incompatibilities. Four mating types were recognized in each of two varieties but the other variety comprised eight mating groups. The members of each group did not mate with each other, but any one of any group would mate with any one of any other group of the same variety. There was no intergroup incompatibility.

Genetical analyses of the progenies of a mating have proven difficult even for the populations that have two and four mating groups. For example, the data available indicate that at least three of the four mating types will give segregations of all four types. Jennings (1942) recognizes that such genetic behavior is not to be analyzed in terms of two allelic series ($S_1S_2Z_1Z_2$ or *AaBb*) of two factors each and he states that "it appears best to await further knowledge before speculating more on the mechanism of the inheritance." Genetical analysis of progenies in the species of *Paramecium* (*P. aurelia* and *P. busaria*) is somewhat difficult for there are complicated internal processes involving nuclear reorganizations of endomixis and autogamy, endo-self-reproduction, and self-differentiation or change of mating type (Jennings, 1941). Further factoral genetic analyses await more definite data on these conditions and processes in relation to the segregations in progenies. These complications are being studied and the results obtained to date have been reported and discussed especially by Jennings, Sonneborn and Kimball.

Incompatibilities in Euplotes patella. In the studies of another ciliate, *Euplotes patella,* Kimball (1943) found no complications of endo-self-reproduction and self-differentiation and hence he was able to analyze the six intra-incompatible but cross-fertile mating groups in terms of "mating type" factors.

In comparison to incompatibilities in bisexual diploid flowering plants there is intra-genotypic cross-incompatibility but complete inter-genotypic cross-fertility.

In Kimball's interpretation there are only three *S* factors (Kimball's mating type factors) in one allelic series. The diploid combinations give three heterozygotes (S_1S_2; S_1S_3; S_2S_3) and three homozygous genotypes (S_1S_1; S_2S_2; S_3S_3). This genetic pattern is like that assumed for the simplest personate incompatibilities in flowering plants except that these reactions are diplont-diplont with each conjugant possessing two *S* factors. Hence the reactions within each conjugant and between them involve relative activities of two factors. There is no opportunity for hidden incompatibility or for the elimination of homozygotes. Although homozygotes occur they are normally not self- or intra-reproducing. The occurrence of homozygotes emphasizes the fact that the incompatibilities are exclusively inhibitions of the conjugation mechanism and are independent of gametic reactions.

The nature of incompatibilities in ciliates. There is not time to-day to review the numerous excellent studies by Jennings, Sonneborn, Kimball and others on various phases of the life cycles and on the features and processes involved in sexual reproduction of the ciliates. It is, however, obvious that incompatibilities inhibit certain matings, particularly those that are intra-genotypic. Thus far it appears that the evaluations of the various investigators consider that the action of the mating factors effect both the successful matings and the failures in mating. It may be suggested that the mating reactions constitute a fundamental feature of intra-specific sexual reproduction and that the incompatibilities are superimposed as modifying factors which effect only the incompatible reactions.

Jennings (1939, page 430) has noted that, in respect (a) to "self-sterility" and intra-group cross-sterility which effect failures in reproduction and (b) to successful inter-group mating which effect reproduction, there is similarity and possible relationship between the "self-sterility" of flowering plants and the so-called "sexuality" of the infusaria.

IV. CONCLUDING REMARKS

1. The present knowledge regarding the extent of intraspecific incompatibilities in the vast array of plants and animals is relatively meagre yet it is sufficiently complete for certain of the species in which it is known to provide a basis for the recognition of the fundamental features.

2. The incompatibilities noted in this presentation are selective limitations to the sexual reproduction of homomorphic hermaphrodites. They limit or prevent both self-reproduction and intra-genotypic cross-reproduction. They effect inter-genotypic intraspecific reproduction.

3. Incompatibilities operate by inhibiting certain of the physiological interactions that are essential to sexual reproduction but which are not features of nuclear or gametic sexuality.

4. Pollen-tube reactions in the pistils of flowering plants, fusions of mycelia and spermatial fertilizations in fungi, and conjugations between individuals of ciliates are all stabilized reactions of intraspecific sexual reproduction. When there are no incompatibilities there is unrestricted interaction in these features of intraspecific reproduction. Incompatibilities are superimposed on these mechanisms and they effect certain failures in interactions which would otherwise occur.

5. Incompatibilities are genetically determined by factors which segregate in meiosis. In the homomorphic bisexual members of a population they may be the only feature of sexuality that does segregate in meiosis. This situation has not been recognized in many considerations of sexuality especially in the fungi.

6. The simplest mode of incompatibilities is that which is determined by a single allelic pair of factors (personate mode) operating in haplont-haplont relations. Then there may be only two mating groups. A minimum number of multiple alleles in a haplont-diplont relation is three. In all the classes there may be numerous multiple factors and numerous mating genotypes. Further complexities involve two or more allelic series (a) of incompatibility factors (amphipersonate) or (b) of one such series in complementary relations with factors which favor fertility (associate mode) or of more than one series of each (amphiassociate).

7. The basic incompatibility reaction is evidently one of independent self-antagonism. But the presence of two or more different factors enforces various types of complementary and competitive reactions.

8. In addition to incompatibilities there are other methods of preventing self-and intra-genotypic reproduction and of enforcing bigenotypic reproduction in hermaphro-

dites. (a) There are herkogamy and dichogamy which operate to separate elements of sexual reproduction previous to their interaction. (b) There are the conditions of polymorphism, especially developed in dimorphic and trimorphic species of flowering plants, in which incompatible reactions are correlated with morphological features.

It is of course to be recognized that in any truly dioecious species the reproduction of the unisexual individuals is obligately bigenotypic.

9. Intraspecific incompatibilities operate with remarkable similarity in phanerogams, in fungi, and in ciliates. Differences in the locus of sexual reproduction in the life cycles of these diverse and phylogenetically widely separated groups of organisms are reflected in three distinct classes of expression and genetic control which may be designated (I) haplont-diplont, (II) haplont-haplont, and (III) diplont-diplont. Without doubt future studies in the groups mentioned and in other groups of plants and animals that were not mentioned will reveal further modes or even other classes of reaction and determination.

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A COMPLEMENTARY LETHAL EFFECT IN THE SEX-LINKED GROUP OF HABROBRACON[1]

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According to the multiple allele theory of sex determination in the parasitic wasp *Habrobracon juglandis* (Ashmead) a single series of allelic factors, designated the x series, differentiates sex (Bostian, 1939, Horn, 1943, Whiting, 1940, 1943). A haploid male may contain any one of the *n* members of this series—xa, xb, xc, etc.; a diploid male is homozygous—xa/xa, xb/xb, xc/xc, etc.; and a female may have any one of the $\frac{n^2 - n}{2}$ possible heterozygous combinations—xa/xb, xa/xc, xb/xc, etc. These alleles, regarded as chromosome segments containing many genes, nevertheless act as units in heredity, indicating lack of crossing over within the segment. Crossing over occurs between the x alleles and various mutant genes both to the left and to the right.

No experimental proof for the multiple genic nature of the x alleles has yet been obtained. It is tentatively assumed as being more probable than a series of single genes because of the complexity of sex differences and because sex in Drosophila and in other forms is differentiated by many genes segregating as a unit (X *vs.* Y).

About one third of the offspring of a mated female are haploid males from unfertilized eggs. If the sex allele of the father (xc) differs from those of the mother (xa/xb), the zygous offspring are all females (xa/xc and xb/xc) constituting a three-allele fraternity. If, however, the father has a sex allele (xa) similar to one of those possessed by the mother (xa/xb), the zygous offspring are females (xa/xb) and diploid males (xa/xa) constituting a two-allele fraternity. Diploid males are of low viability relative to their sisters, so that a 1:1 ratio is not to be expected and there are many nonhatchable eggs.

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Allelism of the series of sex-differentiating factors was shown by their linkage with the mutant gene fused, *fu*, affecting antennae, tarsi, wings. In two-allele fraternities from mothers heterozygous for fused crossed with fused males, about ten per cent. of the females and about ninety per cent. of the diploid males are fused ($\frac{xa\ +}{xb\ fu}$ female by $\underline{xb\ fu}$ male produces 90 per cent. $\frac{xa\ +}{xb\ fu}$, 10 per cent. $\frac{xa\ fu}{xb\ fu}$, 90 per cent. $\frac{xb\ fu}{xb\ fu}$, 10 per cent. $\frac{xb\ +}{xb\ fu}$), or about 90 per cent. of the females and about 10 per cent. of the diploid males are fused ($\frac{xa\ +}{xb\ fu}$ female by $\underline{xa\ fu}$ male produces 90 per cent. $\frac{xb\ fu}{xa\ fu}$, 10 per cent. $\frac{xb\ +}{xa\ fu}$, 90 per cent. $\frac{xa\ +}{xa\ fu}$, 10 per cent. $\frac{xa\ fu}{xa\ fu}$) according as fused is associated with the same sex allele or with opposite alleles in the parents. This rule has been shown to apply for five inbred stocks involving nine different alleles—xa/xb, xc/xd, xe/xf, xg/xh, xa/xi (Whiting, 1943).

In three-allele fraternities sex-linkage of fused is masked because the two genotypically different types of females (xa/xc and xb/xc) are indistinguishable. Fused and non-fused females appear in approximately equal numbers and there are no diploid males.

Horn (1943) made reciprocal crosses between two stocks having different sex alleles (xa/xb and xe/xf). The males were in each case fused. F_2 fused males were backcrossed to females of both parental stocks heterozygous for fused. Each of these F_2 fused males proved to have a sex differentiator from one parental stock only, usually the paternal because the gene fused was of paternal origin. All Horn's data were according to expectation.

The investigation reported in the present paper was originally planned to test the multiple allele theory further by backcrossing the F_1 hybrid females from two stocks with different sex alleles (xa/xb and xc/xd) to males of one of the parent stocks (xa/xb). The initial experiment indicated what appeared to be a lethal effect on certain classes of the offspring from the backcross and it seemed desirable to investigate the nature and location of the lethal factor further as a contribution toward the general problem of genetics in Habrobracon.

MATERIALS AND METHODS

In addition to fused, the gene stubby, reducing number of antennal segments, was used in the experiments because it had been shown to be sex-linked. Hager (1941) showed linkage of fused with stubby and loose linkage of stubby with sex (33.6 to 37.5 per cent. recombinations). He arranged the factors with intervals as follows: x—(8.6 to 17.6)—*fu*—(22 to 24.2)—*sb*.

Sex alleles and origins of the six stocks used in these experiments have been reported (Whiting, 1943). Fused is kept in heterozygous condition because of female-sterility. Formulae of the stock females, including orange eye color, *o*, stubby antennae, *sb*, and fused antennae, *fu*, as well as sex alleles are as follows:

(1) $\frac{\mathrm{o\ xc}}{\mathrm{o\ xd}}$ (11–o) (2) $\frac{\mathrm{xa\ sb}}{\mathrm{xb\ sb}}$ (35–sb) (3) $\frac{\mathrm{o\ xa\ sb}}{\mathrm{o\ xb\ sb}}$

(4) $\frac{\mathrm{o\ xe}}{\mathrm{o\ xf}}$ (5) $\frac{\mathrm{xa\ +}}{\mathrm{xb\ fu}}$ (6) $\frac{\mathrm{o\ xa\ +}}{\mathrm{o\ xb\ fu}}$.

In the experiments orange-eyed females heterozygous for stubby (or for fused) were crossed to black-eyed stubby (or fused) males. Thus the diploid biparental sons could be separated by their black eyes from their haploid orange-eyed brothers.

All progeny which did not emerge from pupal cases were removed with a needle and were classified whenever possible. Eye color, as well as the traits fused and stubby, is separable in all but the youngest pupae.

Experiments with Stubby Antennae

Experiment 1. Orange stubby females, $\frac{o\ xa\ sb}{o\ xb\ sb}$, were mated to orange males, *o xc* or *o xd.* The daughters, designated "F_1 mothers" below, were mated to black-eyed stubby males, *xa sb* or *xb sb,* designated "Fathers" below. Two-allele and three-allele fraternities are to be expected in equal numbers as shown below.

F_1 mothers	*Fathers*	
	xa sb	*xb sb*
$\frac{o\ xa\ sb}{o\ xc\ +}$	two-allele	three-allele
$\frac{o\ xa\ sb}{o\ xd\ +}$	two-allele	three-allele
$\frac{o\ xb\ sb}{o\ xc\ +}$	three-allele	two-allele
$\frac{o\ xb\ sb}{o\ xd\ +}$	three-allele	two-allele

In the three-allele fraternities sex-linkage of stubby is masked, as in the case of fused, because the two genotypes of females are indistinguishable. In the two-allele fraternities a deficiency of stubby females (35 per cent.) is to be expected because they are crossovers, but stubby should be in excess (65 per cent.) among the diploid males because they represent the original combination.

Since the results obtained in the first tests under this experiment proved contrary to expectation, they were repeated twice, the first repetition closely following the original, the second a year later. Different strains of the same stock were used. All agreed in showing a deficiency of wild type offspring.

In the 68 fraternities without diploid males, the females were 2,101 stubby, 1,147 wild type; the haploid males were 374 orange stubby, 469 orange. In the 39 fraternities including diploid males, the females were 482 stubby, 493 wild type; the diploid males were 191 stubby, 49 wild type; the haploid males were 229 orange stubby, 286 orange. Average number of females per fraternity without diploid males was therefore 47.7, but only 25.0 when diploid males were present. Ratio of females to haploid males was 3.85 in the former group, but only 1.89 in the latter. These results are to be expected since only half of the fertilized eggs in two-allele crosses are female-producing.

Studies on hatchability of eggs and viability of offspring (Bostian, 1935) have shown greater fecundity in three-allele than in two-allele crosses. Comparable results are also obtained without observation of eggs by using number of egg-laying days (total days of active adult lives of mothers kept in incubators) as a basis of comparison. In Experiment 1 there were 3,248 females produced in 1,044 egg-laying days (3.11 per day) by mothers with no diploid sons and 975 females in 635 egg-laying days (1.535 per day) by mothers with diploid sons.

Comparison may be made between the three-allele crosses of this experiment and certain other crosses between stocks with different sex alleles (*o/o xc/xd* by *xa sb* or *xb sb*) which produced 483 females in 135 egg-laying days (3.57 per day). Although differences in cultural conditions may have influenced the result, the fact that the three-allele fraternities of the experiment averaged fewer females suggests the presence of a lethal.

Experiment 2. This experiment was similar to Experiment 1 except that the F_1 mothers resulted from the reciprocal cross. The daughters of orange females, $\frac{o\ xc}{o\ xd}$ by orange stubby males, $\underline{o\ xa\ sb}$ or $\underline{o\ xb\ sb}$, were mated to black-eyed stubby males, $\underline{xa\ sb}$ or $\underline{xb\ sb}$. Of the two tests in this experiment, the second, made after the lapse of a year, agreed with the first in results obtained.

In the 15 fraternities without diploid males, females were 454 stubby, 276 wild type; haploid males were 121 orange stubby, 125 orange. In the 15 fraternities including diploid males, females were 142 stubby, 152 wild type; diploid males were 47 stubby, 9 wild type. In this group counts of haploid males can not be used because of presence of the gene fused reducing viability. Among the diploid offspring in this experiment also, there is a marked deficiency of wild type offspring. Females lacking diploid brothers average 48.7 per fraternity, but only 19.6 per fraternity as expected when diploid males are present.

Experiment 3. This experiment was similar to Experiment 2 except that $\frac{o}{o}\frac{xe}{xf}$ females were used instead of $\frac{o}{o}\frac{xc}{xd}$ females. The daughters of orange females, $\frac{o}{o}\frac{xe}{xf}$, by orange stubby males, $\underline{o}\ \underline{xa\ sb}$ or $\underline{o}\ \underline{xb\ sb}$, were mated to black-eyed stubby males, $\underline{xa\ sb}$ or $\underline{xb\ sb}$. Twenty matings were made of which 15 failed to produce diploid males. Since numbers of offspring were rather small, it is probable that some of these 15 involved only two alleles and that diploid males were lacking due to error of sampling.

In the 15 fraternities without diploid males, the females were 316 stubby, 215 wild type; the haploid males were 122 orange stubby, 178 orange. In the five fraternities including diploid males, the females were 47 stubby, 46 wild type; the diploid males were 18 stubby, 2 wild type; the haploid males were 41 orange stubby, 58 orange.

In the former group there are 35.4 females per fraternity, in the latter only 18.6. Ratio of females to haploid males is 1.77 in the former, but only 0.94 in the latter as expected. The ratio of wild type to stubby females is low in this experiment, indicating that the lethal is present in xe/xf material as in xc/xd. In the five fraternities including diploid males, only about half of the females are wild type, instead of the 65 per cent. expected on the basis of 35 per cent. crossing over between stubby and x. In the 15 fraternities without diploid males, stubby females are more numerous than wild type, 1.47 : 1 instead of 1 : 1.

For Experiments 1, 2 and 3, in the fraternities lacking diploid males, ratios of stubby to wild type females are 1.83 : 1, 1.64 : 1 and 1.47 : 1, respectively. Since some of these fraternities in Experiment 3 were probably two-allele, the data are not used in the calculations below.

It is probable that all or nearly all the 83 fraternities of Experiments 1 and 2 lacking diploid males were three-allele because numbers were larger. The fact that wild type were reduced in Experiment 1 even more than in Experiment 2 indicates that no two-allele fraternities were included despite the fact that there were more fraternities lacking (68) than including (39) diploid males. Females in these two experiments totalled 2,555 stubby, and 1,423 wild type, giving a ratio of 1.8:1 for the three-allele fraternities, instead of the expected 1:1. A lethal factor linked with the dominant allele to stubby would therefore allow 36 per cent. crossovers if its action were completely lethal.

In Experiments 1, 2 and 3, ratios of stubby to wild type among the females of the fraternities including diploid males (two-allele) are similar. In the total of 59 fraternities females were 671 stubby, 691 wild type; diploid males were 256 stubby, 60 wild type.

If it be assumed that the factor or set of factors decreasing wild type diploids is similarly effective in the diploid males and females of the two-allele fraternities and in the females of the three-allele fraternities, the crossover value between x and stubby may be found. As stated above the ratio of stubby to wild type females in the three-allele fraternities is 1.8:1 instead of 1:1. The expected number of wild type females and males in the two-allele fraternities may therefore be obtained by multiplying the actual number by 1.8. This gives 1243.8 *vs.* 691 for the females and 108.0 *vs.* 60 for the males. Crossing over as shown by the females is then 35.1 per cent. and by the diploid males 29.6 per cent. The former value is within the range of variations found in experiments not involving a lethal (Hager, 1941). The deviation of the latter is insignificant.

Since crossing over seems to be closely similar between x and *sb* and between the lethal and *sb*, it would be expected that x and the lethal would be closely linked if the latter lies to the left of *sb*. This would very greatly decrease the number of females. Moreover, among the females, the ratio of stubby to wild type or of wild type to stubby should be similar to the ratio of stubby to wild type among the diploid males, according as the lethal were to the right or to the left of x respectively. These conditions are far from being met.

Experiment with Fused

Experiment 4 was planned as a further test of the position of the lethal. Orange females, $\frac{o\ xc}{o\ xd}$, were mated to orange fused males, $\underline{o}\ \underline{xa\ fu}$ or $\underline{o}\ \underline{xb\ fu}$. The daughters, designated "F_1 mothers" below, were mated to black-eyed fused males, $\underline{xa\ fu}$ or $\underline{xb\ fu}$, designated "Fathers" below. Two-allele and three-allele fraternities are to be expected as when stubby was used. The various combinations are shown below.

F_1 mothers	*Fathers* $\underline{xa\ fu}$	$\underline{xb\ fu}$
$\frac{o\ xc\ +}{o\ xa\ fu}$	two-allele	three-allele
$\frac{o\ xc\ +}{o\ xb\ fu}$	three-allele	two-allele
$\frac{o\ xd\ +}{o\ xa\ fu}$	two-allele	three-allele
$\frac{o\ xd\ +}{o\ xb\ fu}$	three-allele	two-allele

Because of sex-linkage and female-sterility of fused and recent derivation of the orange fused stock from the fused stock, it may be supposed that association of fused occurred more frequently with the same sex allele in the F_1 mothers and fathers. Two-allele fraternities may therefore be expected to occur more often than three-allele. There were actually 46 of the former type but only six of the latter.

In the six three-allele fraternities of this experiment, the females were 293 fused, 332 wild type; the haploid males were 120 orange fused, 128 orange. This is close to the 1:1 ratio expected for fused if its somewhat lowered viability be considered.

In the 46 two-allele fraternities, the females were 396 fused, 2,655 wild type; the diploid males were 650 fused, 41 wild type; the haploid males were 1,108 orange fused, 1,110 orange, giving crossover values 12.98 per cent. as shown by the females and 5.93 per cent. as shown by the diploid males. As expected females are almost twice as frequent in the three-allele fraternities as in the two-allele, ratio of females to haploid males being 2.12 among the former, 1.37 among the latter.

In this experiment there is evidently no appreciable effect of the lethal which must therefore be located to the right of stubby.

SEMILETHALS AND THE LINKAGE MAP

Table 1 shows the various combinations expected from a cross of an orange-eyed female heterozygous for stubby and for a pair of complementary lethal factors, L^a/L^b, by a stubby male having one of these factors, L^b. Stubby is associated with the same sex factor, xa, in the female as in the male. The alternative gametic types of eggs combined with the single type of sperm are shown in the second column for the noncrossovers, for the single crossovers in regions 1 and 2 and for the doubles respectively. The third column shows the distribution of the four phenotypes with λ designating the heterozygous combination L^a/L^b. Actual numbers of offspring are shown in the fourth column followed by the ratios of stubby and wild type given separately for the females and for the diploid males.

The ratio of the phenotypes depends upon three things: (1) degree of lethality assigned to L^a/L^b, (2) sex, diploid males being less viable than females, and (3) crossing over and interference, which determine the ratio of genotypes.

In the three-allele crosses of Experiments 1 and 2, the eggs will of course be comparable in type and ratio to those in Table 1, but since the sperm have a different sex factor all zygotes will be female-producing. Only stubby and the lethal will have a perceptible effect.

Following the actual ratios of offspring in Table 1, theoretical ratios are shown calculated on the basis of

TABLE 1

RESULTS FROM CROSSING ORANGE-EYED FEMALES (o/o xc/xa) HETEROZYGOUS FOR STUBBY ANTENNAE (sb) AND FOR A PAIR OF COMPLEMENTARY LETHAL FACTORS (L^a/L^b) WITH BLACK-EYED (+) STUBBY MALES HAVING A SIMILAR SEX ALLELE (xa). ACTUAL NUMBERS AND RATIOS OF WILD TYPE AND STUBBY DAUGHTERS AND SONS ARE SHOWN AND THEORETICAL RATIOS BASED ON THREE-ALLELE CROSSES IF L^a/L^b IS ASSUMED TO BE EITHER COMPLETELY LETHAL OR SEMILETHAL

Mother $\frac{o\ xc\quad +\quad L^a}{o\ xa\ -0.35-\ sb\ -?-\ L^b}$						Father $\underline{+}\ \underline{xa\ sb\ L^b}$					
Crossover regions	Zygotes $\frac{eggs}{sperm}$	Phenotypes of offspring	Actual numbers	Ratios		Theoretical ratios of offspring					
						L^a/L^b inviable Region 2—0.36			L^a/L^b 0.481 viable Region 2—0.10		
				♀♀	♂♂	Total	♀♀	♂♂	Total	♀♀	♂♂
(–)	$\frac{o\ xc\ +\ L^a}{+\ xa\ sb\ L^b}$	+ ♀ λ				0			0.282	0.415	
	$\frac{o\ xa\ sb\ L^b}{+\ xa\ sb\ L^b}$	sb ♂	256		0.810	0.416		0.767	0.585		0.728
(1)	$\frac{o\ xc\ sb\ L^b}{+\ xa\ sb\ L^b}$	sb ♀	671	0.495		0.224	0.489		0.315	0.464	
	$\frac{o\ xa\ +\ L^a}{+\ xa\ sb\ L^b}$	+ ♂ λ				0			0.152		0.189
(2)	$\frac{o\ xc\ +\ L^b}{+\ xa\ sb\ L^b}$	+ ♀	691	0.507		0.234	0.511		0.065	0.096	
	$\frac{o\ xa\ sb\ L^a}{+\ xa\ sb\ L^b}$	sb ♂ λ				0			0.031		0.039
(1, 2)	$\frac{o\ xc\ sb\ L^a}{+\ xa\ sb\ L^b}$	sb ♀ λ				0			0.017	0.025	
	$\frac{o\ xa\ +\ L^b}{+\ xa\ sb\ L^b}$	+ ♂	60		0.190	0.126		0.233	0.035		0.044

0.35 as the recombination ratio between x and *sb*. If the combination L^a/L^b is assumed to be completely lethal, the data from the three-allele crosses give 0.36 as its recombination ratio with *sb*. With these linkage map intervals, expected ratios of stubby and wild type are calculated and given separately for females and diploid males (columns 7, 8, 9). Agreement is very close to the actual ratio in the two-allele fraternities.

These calculations made on the basis of complete inviability of the L^a/L^b genotype disregard the fact that the mothers, which are of this type, are nevertheless viable. It must therefore be assumed that the lethal effect is not complete, that L^a/L^b is semilethal.

Carson (1941), studying linkage and interference in a three-point cross of Habrobracon involving the genes broken, white and stumpy, presented evidence for a semilethal located an appreciable distance to the left of broken and acting like a recessive, killing a proportion of the sons of heterozygous virgin mothers. He states "map position of a semilethal can not be determined since the degree of lethality is unknown. As higher degrees are assumed, the factor would be shifted to the left, toward the locus of a complete lethal; as lower degrees are assumed, the factor would more closely approximate the locus of *bk*."

In Table 1, columns 10, 11, 12 give the ratios of offspring expected if region 2 is only 10 units in length and L^a/L^b is semilethal permitting viability sufficient to make the phenotypic ratio similar to that expected with region 2 thirty-six units in length and L^a/L^b inviable. To the L^b/L^b noncrossovers for region 2 have been added a proportion, p, of the L^a/L^b crossovers for region 2 of similar phenotype and to the L^b/L^b crossovers for region 2 have been added a similar proportion of the L^a/L^b noncrossovers of similar phenotype. In the present instance, p (= 0.481481) is found from $\frac{0.90 + 0.10\,p}{0.10 + 0.90\,p} = \frac{0.64}{0.36}$. This method applies only if coincidence is 100 per cent.

Degree of lethality of a semilethal factor differs widely under diverse environmental conditions and in different stocks. This has been shown in Habrobracon for a number of visible mutant types of low viability. The mutant genes are here semilethals which have a recognizable effect on the surviving individuals. Factors ordinarily designated as semilethals presumably differ from these only because of our failure to find a conveniently visible trait difference.

In Habrobracon with its haploid males, stocks very quickly become pure for modifying factors by lethal selection. It seems likely that thus a balance of factors should be readily attained tending toward greater viability. Relatively disharmonic combinations may be expected to enhance by their cumulative effect the influence of a semilethal among the progeny of hybrid females. A larger proportion of the L^a/L^b daughters than of the L^a/L^b sisters of the hybrid mother might then be inviable.

COMPLEMENTARY LETHALS IN OTHER FORMS

Complementary lethal effects are found in many hybrid embryos resulting from species crosses. Syngamy and cleavage mitoses may be normal, but disharmonic developmental processes occurring later result in death. Complementary lethals are also noted in intervarietal crosses and in certain instances the factorial basis has here been analyzed. Thus Wiebe (1934) has shown that F_1 seeds resulting from crosses of two barley varieties germinate normally but the hybrid embryo usually fails to emerge or the plumule dies. The F_1 generation from crosses of certain wheat varieties likewise is inviable (Caldwell and Compton, 1943, Heyne, Wiebe, Painter, 1943) but here the hybrid plants develop normally to the two-leaf stage. Progressive necrosis of the leaves then takes place resulting in death. Both in barley and in wheat it was shown by crossing to other varieties and subsequent crossing of the offspring that the lethal section was caused by the presence of the dominant alleles of two independently segregating pairs. Neither dominant alone, whether homozygous or heterozygous, was lethal. Other hereditary factors occur in wheat causing necrosis which is nonlethal to the plant or semilethal.

Sears (1944) has shown complementary lethal effects in intergeneric hybrids involving Triticum. A single-factor difference may exist between two varieties of a species with one allele determining inviability in the generic cross, but there may be "several factors from each species, all necessary for inviability of the hybrid."

In *Drosophila melanogaster* Schultz (1929) has shown complementary lethal and semilethal effects in the combination of certain Minutes with different Delta alleles and with Jammed. Muller (1940, p. 196) states "It is so common an experience as not to occasion any remark to find that two or more mutant genes, whose viability is not noticeably low when in separate stocks, give a stock whose viability is very much reduced—more than additively—when in combination."

In the example under consideration in Habrobracon, the factor L^a may be represented as two closely linked elements, L_1 l_2, a dominant and a recessive, while L^b contains the alternatives, l_1 L_2. No effect takes place in the azygous male or in the homozygous male or female, but in the heterozygote the cumulative effect of the two dominants, supplemented by modifying factors segregating in oogenesis of the mother, results in disharmonic development with lethal threshold.

SUMMARY

In five tests involving reciprocal crosses of two orange-eyed stocks and in one test of one of these stocks having the loosely sex-linked gene stubby with a third stock, lethal effects were present associated with the dominant allele to stubby. The F_1 females were crossed with black-eyed stubby males closely related to the orange stubby parental stock. In all cases there occurred a marked deficiency of the wild type diploid offspring, both among the males and among their sisters as well as among the females of the fraternities involving three sex-alleles in which there are no diploid males. Since no corresponding decrease occurred among the non-stubby haploid males and since the stocks were apparently breeding true for the "lethal" factors involved, the lethal effect is regarded as complementary.

Failure of association of the lethal with sex was shown in the fraternities involving two sex alleles in which the zygous offspring are differentiated into males and females. Moreover a test similar to those involving stubby but made with the closely sex-linked gene fused in a closely related stock failed to show any lethal effect. It is then probable that the lethal factor lies to the right of stubby.

Thirty-six per cent. recombination between stubby and the lethal would occur in case of complete inviability. With about 50 per cent. viability, recombinations would be 10 per cent. Semilethality is assumed, fluctuating because of multiple factors which segregate in oogenesis of the hybrid females.

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CURRENT CONCEPTS OF PLAY IN ANIMALS

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PRESENT-DAY understanding of animal play is regrettably limited, and current views on the subject are considerably confused. On the one hand, there are the hundreds of observations made by naturalists, by animal breeders and by nearly every one who has kept a household pet to indicate that animals of many species do exhibit various types of behavior which, if they were observed in humans, would undoubtedly be called play. On the other hand stands our undeniable ignorance as to the essential nature of play, its causes and its results. The richness of the observational evidence is in sharp contrast to the poverty of scientific knowledge.

It is significant that authors of several recent American text-books on comparative or physiological psychology have failed to make any mention of play in animals, or have skipped over the subject as lightly as possible (Warden, Jenkins and Warner, 1936; Maier and Schneirla, 1935; C. T. Morgan, 1943). Their reluctance or inability to treat the topic undoubtedly derives from the exclusively observational character of available data and the obvious inadequacy of existing interpretations.

It is not the purpose of this paper to present new data pertinent to the problem under consideration, or to propose any original, all-inclusive theoretical interpretation. Instead it is hoped that a survey of existing knowledge and an evaluation of the theories presently available may point the way toward profitable lines of future work which will lead to a more complete understanding of the subject.

COMMONLY ACCEPTED CHARACTERISTICS OF PLAY

Play is generally regarded as differing from non-playful activities in several ways. Whether or not it is possible to discern any pervasive characteristics common to all forms of behavior which are commonly listed under this rubric depends upon the definition of play. At this point, however, we shall overlook this source of difficulty and list the outstanding characteristics of playful behavior as set forth by most students of the subject.

(1) It is commonly stated or implied, although it can not be objectively demonstrated, that playful behavior in animals as in man carries an emotional element of pleasure. Not all pleasant activities are playful; but all play is assumed to be pleasurable. (2) Play is usually regarded as characteristic of the immature animal rather than the adult. Grown individuals may play; but they do so less frequently than juvenile members of their species. (3) It is usually supposed that play differs from non-playful responses in having no relatively immediate biological result which affects the continued existence of the individual or the species. In other words, play is customarily regarded as non-utilitarian. (4) The outward forms of play are relatively species-specific. Dogs play in certain ways, horses in others, and apes in still others. (5) The amount, duration and diversity of play in a given species is related to its phylogenetic position. In general, play is more frequent, more variable, and occurs during a longer portion of the life span in higher animals than in lower. The play of fishes appears infrequent and stereotyped when compared with that of lower mammals; while the play of dogs is less diversified and prolonged than that of monkeys and apes.

TYPES OF BEHAVIOR WHICH HAVE BEEN CALLED PLAY

A wide variety of animal responses are frequently lumped together under the general heading of play. The categories are not clear cut, but may merge insensibly one into another; and sometimes several kinds of play may be combined in an integrated series of reactions. In listing the main types of play we shall temporarily refrain from any evaluation of the validity of the classification.

General bodily activity. Many young animals display a great deal of bodily activity, chiefly of a locomotor character, in which vast amounts of energy are expended and no obviously useful result is achieved. Fishes of some species periodically leap above the surface of the water; some birds indulge in elaborate aerial acrobatics; colts gallop about the pasture; puppies race back and forth in the yard; kittens scamper wildly across the room over the sofa and under the chair; in addition to climbing, sliding and jumping, chimpanzees execute complicated bodily actions such as turning somersaults, or revolving rapidly in pinwheel fashion while lying at full length on the ground (Kohler, 1931). Some writers regard these reactions as a form of play (Mitchell, 1912; Haigis, 1941; Gudger, 1944).

Youthful practice of adult activities. Young animals of many species exhibit various behavior patterns which also appear in the repertoire of the adult individual; but when executed during immaturity the responses are often incomplete, and seem to lack any practical outcome. Well-known examples of such behavior, which is generally interpreted as play, are the mock battles of puppies, the pursuit and capture of any small, moving object by kittens and the infantile or juvenile sex reactions of many species (Groos, 1898).

Exploration and experimentation. Responses falling in this category may range from very simple investigatory reactions to highly complex and original types of experimentation (C. L. Morgan, 1900). The tendency of young birds and mammals to peck, scratch, claw, pull and bite at objects in their environment is illustrative of the relatively simple exploratory reactions which some observers regard as play. In contrast stand the knot-tying and pole vaulting of chimpanzees, which are also examples of manipulative or experimental behavior albeit of a higher order.

Social responses. Certain inter-individual reactions which seem to lack any practical outcome are often listed as play. The "tilting matches" of young British warblers (Pycraft, 1912), "king-of-the-mountain" games of lion cubs (Cooper, 1942), "follow-the-leader" games of young monkeys (C. L. Morgan, 1900) fall in this general category.

Social play is prominent among chimpanzees according to the descriptions of Kohler (1931). He states that when several young apes were given a large sack, one crept into the container and the others pulled it about on the ground. Small chimpanzees sometimes rode pickaback on the shoulders of older animals and then slid downward, grasping the bearer's trunk with the hind legs and walking on the ground with the hands.

The "dancing" of young apes would probably come under the heading of social play. This activity is highly variable and ranges from spinning round and round in a solo performance to participation in rather complicated group performances. Several chimpanzees may start to march single file around a post or box. Gradually their pace increases and they trot, often with emphasis upon one foot so that a primitive kind of rhythm emerges; and as they trot and stamp, the animals may wag their heads in time with the pedal rhythm. A direct quotation from Kohler will serve to illustrate the complexity and group character of the behavior.

> As the whole group were joyously trotting round a box, little Konsul stepped to one side outside the circle, drew himself up to his full height, swung his arms to and fro in time to the trotting, and each time that fat Tschego passed him, caught her a sounding smack behind.

Inter-species reactions. Although animals of one species occasionally respond to those of a foreign species in what might be regarded as a playful manner, play between species is not common. The outstanding exception to this generalization is the play which takes place between man and his pets.. If one is to accept any of the characteristics or criteria of play which are accorded common usage, it must be admitted that the dog plays with his master (Russell, 1936). The animal may engage in sham hunts or battles with the man in much the same manner as with another dog. On the other hand he may with equal readiness participate in a "learned" game such as "fetch," or perform various tricks without any material reward. It is particularly significant to note that the animal may take the initiative in beginning the interspecies play, and signalize in a variety of ways his eagerness to tease his master into a game. In similar fashion young chimpanzees play with human companions (Wolfe, 1936); and the desire to do so is so strong that opportunity to play has successfully been used as an incentive in certain learning experiments (Yerkes and Petrunkevitch, 1925).

CRITERIA AND CAUSES

The majority of interpretations purporting to define or explain play are speculative in nature, deductively derived and completely untested.

Surplus energy. The poet Schiller is reputed to have suggested that play is an expression of overflowing energy which can find no other outlet. Actually, as Curti (1930) has pointed out, Schiller merely noted that playful behavior usually occurs when an ample supply of energy is available; but later writers have reinterpreted the original suggestion to form a theory stating that certain types of play constitute a release of extra physical, and perhaps mental or emotional energy.

A variation of this hypothesis, in modern dress, has been restated by Tolman (1932) in the suggestion that men and perhaps some of the lower animals have a need under certain conditions to achieve "mild harmonious fatigues." It is proposed that when the organism is in a "neutral sort of metabolic condition," and other needs or appetites are not strongly engaged, there is a condition of unspent energy. This state is regarded as one of physiological disequilibrium requiring complementary fatigue for neutralization. In defining play, Tinklepaugh (1942) states as one criterion the fact that play is a type of activity which occurs under conditions of surplus time and energy.

Suggestions of this sort have little to recommend them. In the first place they are based upon the most obvious sort of circular reasoning. The catch lies in the definition of the term "surplus." When a cat chases, catches and devours a mouse, a certain amount of energy is expended; but no one suggests that this is extra, or surplus energy. Now, when the same cat chases, catches and chews on a rubber ball, an equal energy loss may occur; but in this case it is said to be surplus energy which has been released. Catching and eating mice is serious business for the cat (or at least the human observer thinks that it *should* be); whereas pursuit of a rubber ball serves no obvious, immediate and practical end (in so far as the observer can tell). Therefore ball chasing must be play, whereas mouse chasing is not play. Refraining for the moment from commenting upon the anthropomorphic reasoning involved, we can see that the decision as to whether or not the expended energy is surplus energy, depends upon the interpretation of the behavior as playful or serious. Therefore to set up as one criterion or explanation of play the condition that it involves the release of surplus energy is to do no more than complete the circle.

As far as mental and emotional energy are concerned, our concepts in this field are so fuzzy and debatable that it is sheer nonsense to predicate explanations of behavior upon the supposed accumulation and discharge of these hypothetical forces. Definition of one unknown in terms of a second unknown is good algebra but poor psychology.

Present-day knowledge of physiology does not support the belief that physical energy is something which can be stored up in the organism like water in a reservoir. There is no known process whereby unexpended energy "backs up" and creates a pressure, demanding release. To be sure certain sources of potential energy, such as liver glycogen, may be accumulated; but this is quite a different matter; and, thus far at least, no one has suggested that play occurs because the liver feels the need of discharging stored glycogen.

Instead of referring to energy, surplus or otherwise, it seems best to conceive of a muscle, nervous unit or organism as being either completely rested and ready to respond maximally to stimulation, or as being partially or almost completely exhausted, in need of rest and repair. Under conditions of partial or total exhaustion the organism may be expected to react weakly, incompletely, or to fail entirely to respond to stimuli from the external environment. Such a concept would lead us to anticipate that play, which, like any other type of behavior, constitutes a response to stimulation, would occur most frequently and readily when the animal is rested and in a state of readiness to react. This is exactly what happens; but be it noted that non-playful activities also occur most frequently under the same conditions. Thus, the tired animal is less apt to play than is his rested fellow; but he is equally less likely to "work."

Several writers, including Mitchell (1912), have objected to the interpretation of playful activities as the mere discharge of a waste product; and have insisted that the energy employed in play is expended upon "the business of youth." C. L. Morgan (1900) also states that "normal" rather than "surplus" energy is involved in the play of animals.

Finally, as Groos (1898) and others have observed, superabundant energy is not always a condition of play. Young animals can often be seen to play to the point of apparent exhaustion, lie panting with fatigue, and suddenly respond to the advent of a play-inducing stimulus with the abrupt resumption of their energy-draining games.

It is generally recognized that young animals are often more active than adult members of their species (Cooper, 1942), and perhaps the observation of this difference has given illusory support to the surplus energy criterion of play.

General exuberance, or joie-de-vivre. It is often stated that playful behavior is "enjoyed for purely its own sake" (Tinklepaugh, 1942), "expresses a joy of living" or "manifests a general exuberance" (Pycraft, 1912). Some writers combine this criterion with the surplus energy criterion, and conclude that play grows out of a pleasure in being active (Haigis, 1941). From this point it is but a step to the conclusion that animals play because playing is pleasant.

Before criticizing this theory let us state unequivocably that the comparative psychologist does not look upon all animals other than man as machines which automatically carry out their life functions without intelligence, without conscious purposes and without any emotional involvement. On the contrary, many if not all of the lower mammals, the birds, reptiles, amphibians and fishes manifest varying degrees of intelligence and adaptability. To be sure, their behavior is less variable than that of man, and their ability to profit from experience may be relatively limited; but many observers believe that the differences are more of degree than of kind. There is, furthermore, little reason to deny to animals various kinds and degrees of emotional experience. Accordingly, the most militant and objective "behaviorist" can not seriously object to the statement that the dog which romps through the snow, barking, leaping, dashing wildly hither and yon, or plays at "fetch" with his master, is enjoying the experience.

Having done our feeble best to disarm the critical reader who promptly and scornfully cries, "Mechanist!" at any one daring to speak out against anthropomorphic interpretations of animal behavior, let us proceed to examine the purported explanation of animal play as a form of activity which is engaged in solely for the pleasure it brings the performer.

The first and most obvious objection is that this so-called interpretation does not interpret nor explain. It is directly comparable to the explanations of sixteenth century theologicians who interpreted all natural phenomena as manifestations of divine will, or to the animalistic beliefs of more ancient "explainers." One might with equal accuracy state categorically that a plant grows toward the sun, a cloud moves across the sky or a stick floats on the surface of the stream, because the plant, the cloud and the stick derive pleasure from behaving as they do. Here is no explanation, no advance in the understanding of basic causal relationships responsible for the outward activity.

Explaining a playful response by referring its occurrence to one (assumed) character common to all forms of play is equivalent to explaining that water boils because it is hot. To be sure, boiling water is usually quite hot (although its temperature may vary considerably as any tenderfoot who has tried to cook at high altitudes will ruefully attest); but the explanation for boiling is to be found in the elucidation of the relationship between the atmospheric pressure and the vapor pressure of the liquid.

Another indictment against the *joie-de-vivre* hypothesis as an explanation of animal play is that its uncritical adoption tends to discourage and render apparently unnecessary any further attempt to examine in detail the real nature of the reactions thus "explained."

Non-utilitarianism. In defining play the majority of writers have agreed that one of the criteria for this form of behavior is that it is not directly useful, although the possibility of eventually practical results is not denied. There are several practical and theoretical objections to this criterion.

Very often the conclusion that a particular act performed by an animal has no directly practical value is actually no more than a confession of the observer's ignorance. An example will illustrate this point. Various fishes, including members of the family *Belonidae,* exhibit a curious habit of "leaping" over free-floating objects such as sticks and straws. As described by Breder (1932) for *Tylosurus raphidoma* the reaction of the fish is as follows:

> First the fish will swim up slowly to the stick so as to be nearly at right angles to it (in a horizontal plane) and gently protrude the beak through the surface of the water, sliding the tip over the stick. Usually if the stick is too small and gives way too easily, or too large and gives way too little, the fish will withdraw. If it is of the proper buoyancy and sinks ever so little under the weight of the beak, a violent tail action follows and the fish clears the water, but in such a manner that usually part of the body rubs against the stick in passing and the fish falls to the other side, from which it may turn and leap back again.

Many naturalists who have seen this behavior have concluded that it is a playful response lacking in any practical outcome (Gudger, 1944). Other observers have noted that fishes which persistently leap over floating sticks are often heavily infested with ectoparasites that are easily scraped off. It has been suggested that leaping across solid objects may serve to dislodge the parasites, and thus to free the fish of the infestation (Breder, 1932). The validity of this surmise has yet to be established by additional observation or by experimental approach; but the significant point would seem to be that it is susceptible to objective verification or disproof. In contrast, it is a very difficult if not impossible task to establish beyond reasonable doubt the absence of utilitarian function for any type of animal response.

Adoption of the non-utilitarianism criterion in its pure form reflects a certain degree of naiveté. As has been noted, young animals often perform, incompletely, various actions which will be executed in their totality during adult life. In the mature animal the behavior in question can be shown to serve an obvious and biologically useful end, such as mating and reproducing, securing food, self-defense, etc. In the young animal the behavior pattern does not terminate in the same result. Accordingly it is sometimes concluded that the reactions of the youngster are without any immediate result or purpose.

This is obvious sophistry. There is no reason to assume that a given pattern of behavior must serve one and only one end under all conditions and at all stages of development. The flight of a bird may assist it to elude a predacious foe, to secure necessary food, to charm its mate, to transport it to a different environment or to bring about any one of several other equally "practical" results. Furthermore, the scientific study of animal behavior, if it has taught us nothing else, should have impressed upon us the folly of trying to define *a priori* the motives and ends of animal conduct. It is unadulterated nonsense to assume that, in the absence of intensive and prolonged study, the human observer is capable of discerning the presence or absence of a "useful" result consequent to a complex response. (The eating of bones by pregnant female mammals of non-carnivorous species was thought useless until advanced understanding of the physiology of pregnancy revealed the need for extra supplies of calcium.)

The practicality or usefulness of a course of action is a function of a multiplicity of variables. A man transported suddenly into a new and strange cultural setting would be at a loss to recognize the motives for certain response patterns, and might be quite incapable of discriminating between utilitarian and non-utilitarian modes of conduct. The building up of a comfortable financial reserve in anticipation of future emergencies is a practical and utilitarian bit of behavior in our culture. The same behavior would be highly impractical not to say socially dangerous among Mexicans of the Sinaloa region; for in their culture a man is responsible for the support of all relatives whose resources are inferior to his own. Therefore the individual with wealth or a new house is perpetually parasitized by less fortunate relatives who continue with him as long as the money lasts or the house stands. Thus the accumulation of "excess" worldly goods is practical in one social setting and quite the opposite in another.

If, without extensive study, man can not differentiate between "useful" and "useless" behavior in fellow members of his own species, how can he expect to make such a discrimination in connection with the reactions of animals of an entirely different genus, and even class?

Results of Play

Although many writers agree that playful acts must be performed because of the pleasure or satisfaction which they engender, and can not have any immediate, utilitarian outcome, it is nevertheless often stated that certain types of play do possess important, long-term effects.

Youthful practise for adult life. It is common knowledge that immature animals frequently perform incompletely or imperfectly, and without apparent useful outcome, certain patterns of behavior which will be exhibited during adulthood in complete and biologically effective form. This observation has so impressed many writers that they interpret one function of the play of young animals as "an irresponsible apprenticeship to the serious business of life" (Pycraft, 1912; Mitchell, 1912).

Adherents of this view usually make several basic assumptions. (1) The type of play to which this theory pertains is held to be determined largely by heredity, since it is believed to appear without opportunity for learning or imitation, and takes approximately the same form in all members of a given species. (2) Although instinct accounts for its broad outlines, the response is regarded as being imperfectly organized upon a purely genetic basis, so that each individual must perfect the inherited reactions by practice and repetition. (3) An intriguing ramification of the theory is stated in the further postulate that the types of behavior under consideration are all of great biological importance, since each is essential to the life of the individual and thus to the perpetuation of the species. It is pointed out that the play period of infancy permits the practice of these vital responses under conditions where error and incompleteness are not fatal. The extension or modification of inherited tendencies is thus possible before the animal is subjected to the exigencies of an independent existence, wherein the forces of natural selection are constantly operating to weed out inefficiency, and the penalty for error may be sudden death (C. L. Morgan, 1900).

It is not impossible that experience gained during the practice of certain activities during youth increases the efficiency with which the same responses will be performed in maturity. However, the observation that the acts are performed incompletely or inexpertly by the young animal, and appear in more complete and biologically-effective form in the behavior repertoire of the adult, does not justify the conclusion that the perfection of the adult's reaction is a result of the practice during immaturity. If we are to prove, rather than assume, that the kitten's repeated chase and capture of the ball of string is later reflected in improved mousing, it must be shown that kittens which have had no opportunity to play at chasing and catching are poorer mouse catchers in adult life than are other cats which have had normal opportunities for such practice during kittenhood. The same comparison holds true for all other types of play which have been assumed to "polish and perfect imperfect instincts."

Unfortunately, objective proof of this nature is almost completely lacking.[1] Perhaps a puppy which is kept in isolation from all other animals, and thus prevented from indulging in sham fights, will, upon the attainment of maturity, be equally as ferocious and successful a fighter as other dogs which participated in countless mock battles during puppyhood. These suggestions run counter to the interpretation of play as a form of essential training and preparation for adult life; but the important point is that we simply do not know which supposition most closely approximates the facts. Only direct test will settle the question.

In a few instances there is good reason to believe that the "playful" execution of a behavior pattern during immaturity is without effect so far as the performance of the same response in adulthood is concerned. For example, during their wrestling, chasing and mock-fighting immature rats often execute large portions of the adult mating pattern (Small, 1899; Beach, 1942a). If, however, rats are raised in individual cages, so that they have no opportunity for any such practice, they mate during adult life in exactly the same fashion and with the same effectiveness as do their brothers which have been raised with females (Stone, 1922; Beach, 1942b).

This is not to say that the infantile practice of various behavioral reactions is always without effect upon the efficiency with which the responses are executed later in life. The conditions under which a kitten is raised have a powerful effect upon its tendency to kill rats (Rogers, 1932; Kuo, 1938). Some kittens fail to develop rat-killing behavior when raised in isolation but display such reactions after they have observed rat-chasing and killing on the part of another cat (Kuo, 1930).

The play period of some primates is prolonged, and involves the execution of many activities later indulged in by the adult (Yerkes and Tomlin, 1935). It is known that sexually inexperienced adult male chimpanzees often are incapable of mating effectively with the receptive female. Apparently a great deal of experimentation and practice is necessary before this vital part of the animal's behavior repertoire can be carried out efficiently; and Bingham (1928) has found that immature chimpanzees exhibit sex play very frequently during childhood. It is not too much to assume that this practice has its beneficial effects upon adult performance. However, to assume is not to know, and in this case, as in every other, direct test of the hypothesis offers the only means of arriving at a final answer.

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[1] As a matter of fact, young kittens which "play" with rats are less likely to become rat killers when they mature than are kittens that fail to exhibit mock chases or capture responses (Kuo, 1930).

A point sometimes overlooked by adherents to the theory that play serves a preparatory function is the occurrence of similar responses in adult animals. Gooper (1942) has described the play of mature African lions as consisting chiefly of hiding behind objects and then creeping forth to stalk and perhaps leap upon another adult. Presumably the grown animal has no need to practice such activities (although it would undoubtedly be suggested by some authors that this behavior is a means of keeping the responses in good working order!); but of course the fact that such playful reactions occur in adult life does not prevent them from serving a preparatory function in the juvenile animal.

Exploration and experimentation. Exploratory play is sometimes held to familiarize the animal with the properties and potentialities of the world about it. An eventually practical outcome of such play has been thought to lie in its tendency to promote the development of new modes of conduct and to inhibit biologically dangerous reactions. From the psychiatric point of view, Masserman (1944) has suggested that exploratory responses represent one expression of an anticipatory need to determine the relative safety and potentialities of the environment. Although these assumed functions of exploratory play are based upon *a priori* reasoning more than controlled observation, there are some instances in which they can be shown to exist.

Kohler (1931) reports that captive chimpanzees given stout sticks or poles soon devised a crude form of vaulting or jumping, using the stick as a crutch. Originally this response was indulged in apparently as a form of amusement, and was not used as a means of achieving any secondary objective as far as the observer could determine. Although further test and observation would be necessary to establish the point, it seems probable that the pole-vaulting response was a form of play, and the outgrowth of experimentation with the stick. Later, however, when the apes were confronted with the problem of obtaining a banana which was suspended above their reach they quickly put the vaulting technique to good use, employing it as a means of getting the fruit.

Socialization. Some kinds of play have been assumed to encourage particular types of inter-individual reactions. It will be recalled that chimpanzees engage in various social activities which involve participation by several individuals. Tinklepaugh (1940) has proposed that such behavior early in life enhances the individual's adaptability and cooperativeness, both of which are essential characteristics of the species.

Self-expression or diversion. Yerkes (1943) states that play in chimpanzees is one form of self-expression, self-amusement or diversion. He has observed animals draping themselves and wearing strips of cloth or paper, fruit skins, flowering plants or leafy branches. (Whether or not such reactions can be interpreted as self-adornment is a moot point; but the element of diversion is held to be obvious.) Apes often "paint" or smear objects or flat surfaces with feces, and this too is a form of activity which Yerkes regards as self-expressive, and perhaps playful.

Kohler (1931) describes the manner in which chimpanzees moistened white clay with saliva and then proceeded to daub the resulting mixture on their own bodies and upon all sorts of objects in their environment.

The proposed socialization and diversion functions of certain kinds of play appear reasonable and logical, but must be regarded as speculative until further evidence is forthcoming.

POTENTIAL METHODS OF APPROACH

If it is apparent that current understanding of play in animals is limited, and that presently accepted criteria and explanations are inadequate, it should be equally obvious that there are various steps which could be taken to improve the situation.

Controlled observations. The most pressing need is for controlled observation of those activities which are customarily regarded as play. To replace the anecdotal, impressionistic or romanticized descriptions of animal behavior there must be provided detailed, objective accounts based upon repeated observation. Information is needed, not only concerning the observable reactions of the animal, but also regarding various aspects of the environmental situation. So far we know practically nothing as to the probable stimulus patterns which elicit playful responses.

A beginning in this direction has been made by a few workers. For example, Cooper (1942) reports that the play of lions occurs most commonly following a major change in the environment, such as release of the animals from an inside cage, the introduction of new individuals into the pride or the sudden appearance of the attendant. It is added that play frequently takes place just before feeding time, is more common on cool days, and is shown by females more than males.

Studies of the ontogenetic development and regression of play in a single species would be valuable, and would supplement the vague generalization that play is essentially an infantile activity.

In so far as possible records gathered under field conditions should be checked in a setting permitting systematic variation of the stimulus situation.

Definitions and criteria. In attempting to define or set up criteria for play it should first of all be recognized that no single hypothesis can be formulated to explain all forms of play in every animal species. The types of activity which are commonly termed playful are so variable in form and complexity that a different interpretation is indicated at least for each major category. Secondly, it must be apparent that many playful and non-playful pursuits differ in subtle and elusive fashion. There is no sharp borderline between play and work for animals, any more than for man. The two types of activity are often difficult to distinguish, and some complex responses may be partly play and partly work (Kollarits, 1940).

This means that any serviceable definition of play must be based upon a number of predominating characteristics which combine to set if off from non-playful behavior; and can not be derived from adherence to one or two rigid criteria such as imperfect, juvenile performance or non-utilitarianism. It may eventually prove helpful, if the facts warrant, to state that playful reactions as a class tend to lack the immediate, biologically useful results which normally accompany non-playful responses; but it is definitely inadvisable to set up as an inflexible rule the dictum that no activity is play if it achieves a useful end.

Thirdly, the conclusion is inescapable that play must eventually be defined in objective terms. If no such definition has yet been forthcoming its absence may be traced directly to the lack of essential data. In connection with other, equally complex types of animal behavior, workers are building interpretations founded on recognized, demonstrable physiological and psychological processes and functions. Learning, maternal behavior, courtship and mating, territory defense, social dominance and a host of equally important categories of response have proven susceptible to an objective approach. There is no reason to believe that play is unique in this respect.

Experimental validation of theories. We have previously indicated the necessity of subjecting to direct test certain currently accepted interpretations of the functions of play. There is no need to limit ourselves to speculation in this field. If they actually exist, the "preparatory" function, the "experimental" function and the "socialization" function which have been assigned to play can be objectively demonstrated, quantitatively measured and operationally defined. Furthermore, as new evidence justifies their formulation, new theories can and should be exposed to the "test-revise-retest" process of critical analysis.

Human applications. In closing it may be worth while to comment on the fact that play in humans, although it has been studied more extensively than play in animals, is only partially understood (Alverdes, 1932; Blumenfeld, 1941; Britt and Janus, 1941; Curti, 1930; Dever, 1917; Watson, 1919). An evolutionary approach has proven fruitful in advancing our knowledge of many phases of human behavior; and it is not too much to hope that the careful study of animal play will offer potentially significant results in the increased understanding of similar behavior in man.

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THE ANGLER-FISHES, *LOPHIUS PISCATORIUS* ET *AMERICANUS*, USE THE LURE IN FISHING

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LOPHIUS is found in the North Atlantic, off the Atlantic (and Mediterranean) shores of Europe and off those of Eastern North America. Linnaeus early gave the European fish the name *L. piscatorius,* and the American form has been thought to be identical and was from the first given the same appellation. However, as early as 1837, Valenciennes found that the American fish differed from the European form in certain external features—in the teeth, lower lip and the presence of small eye-like spots on the back. These unlikenesses led him to propose for the Western Atlantic fish the specific name, *americanus.*

However, the findings of Valenciennes were considered too insignificant for this species differentiation, and the names *L. piscatorius* was affirmed the correct one until recently. In 1928 and in 1929, irrefutable evidence was produced leading to a return to the name given by Valenciennes to the American fish.

In 1928, Marshall and Grafflin, by microscopical studies of the kidneys of adult specimens from Europe and the United States, found totally unlike structures in certain parts of these organs. And in the following year (1929) Berrill, studying the embryology of the American form, found that the eggs are smaller than those of the European fish. More marked differences were found in the development of certain relatively identical stages of the larvae of the two forms. These clearly demonstrated differences necessitate a return to the name given by Valenciennes, and make it necessary that the feeding habits of the two forms be considered separately.

THE EUROPEAN ANGLER, LOPHIUS PISCATORIUS, USES ITS LURE IN FISHING

As may be seen from Yarrell's figure (1841), reproduced here as Fig. 1, Lophius is large forward (length and breadth of the head being about equal), and falls away rapidly behind to a relatively insignificant tail and caudal fin. Because of this tadpole-like make-up, our fish was called *Rana piscatrix* (fishing frog) by the ichthyologists of the 1500's. The head is very depressed and the large staring eyes have been brought close together onto its flat top. The huge mouth (nearly equal in width to the head) is terminal and somewhat uptilted, and both jaws are thickly beset with long, pointed, backwardly-slanting teeth. Standing on the median line of the head, the anterior dorsal spines have become long, slender and flexible, and the two foremost ones have been transferred forward and anchored just above the upper jaw. These two whiplashes are provided with muscles at their bases, and by these can be moved about in any direction. The anterior one has at the tip a fleshy lappet or "bait"—here shown as forked.

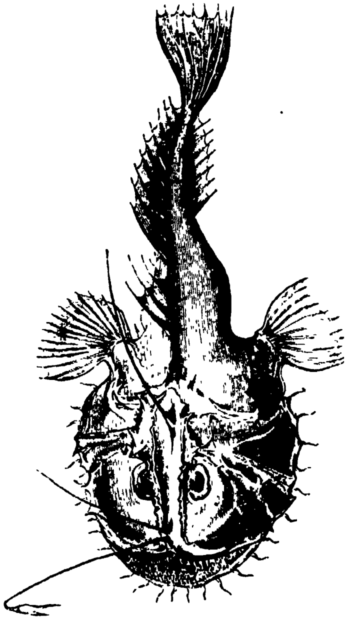

FIG. 1. The Angler-fish, *Lophius piscatorius*, an animate piscine fish-trap.

Yarrell's figure is not the best I know, but it shows the flat wide head with the dorsally placed eyes and the tentacles located almost on the upper jaw. And, better than any other accessible figure, it shows the anterior tentacle with its bifid lure hanging on front but to the right of the center of the mouth with its armament of teeth. From this it will be seen that *a priori* the angler looks to be one of the best animate fish traps in the piscine world.

For our earliest knowledge of the angling habits of this fish, we must go back to the father of natural history, Aristotle (384–322 B.C.). Aristotle must have known the fish at first hand, since he so accurately describes its make-up and its actions. I quote from Sir D'Arcy W. Thompson's translation ("Historia Animalium," Oxford, 1910).

After the general remark that "In marine creatures, also, one may observe many ingenious devices adapted to the circumstances of their lives," then for our fish Aristotle continues thus:

> The accounts commonly given of the so-called fishing frog are quite true. . . . The fishing frog has a set of filaments that project in front of its eyes; they are long and thin like hairs and are round at the tips; they lie on either side, and are used as baits. Accordingly, when the animal stirs up a place full of sand and mud and conceals itself therein, it raises its filaments and, when the little fish strike against these, it draws them underneath into its mouth. . . . Furthermore, the fishing frog is unusually thin when he is caught after losing the tips of his filaments.

The reader will note the significance of Aristotle's final sentence. It shows in what thoroughly scientific fashion he studied this fishing habit. This final statement definitely "clinches" the matter that the lure is of especial use in the catching of food.

From Aristotle's day to the present (a time-span of over 2,000 years), people (scientific and otherwise) have quoted Aristotle only to cast doubts on the accuracy of his observations. From a study of the make-up of Lophius, all are agreed that *a priori* he must catch fishes by the use of the lure. But, since Aristotle, until very recently no man—scientific or lay—could say, "I have seen the angler-fish angle with his lure." And because no one had actually seen it, Aristotle's account was at the best doubtfully accepted. But it seems incredible that in all these long years no one, out in open water or in an aquarium tank, has seen Lophius fishing with the lure.

But at long last *Lophius piscatorius* has been observed while doing this very thing. W. C. Chadwick has seen and briefly described (1929) the feeding "on many occasions" in the tanks of the Marine Biological Station at Port Erin, Isle of Man. Some living young specimens of the coalfish, *Gadus virens,* were introduced on many various days into the tank with the Lophius. Here were its reactions as recorded by Chadwick:

> These [fish] would soon be noticed by the angler, which, while remaining stationary with closed mouth, raised the lure from its horizontal position along the back and jerked it to and fro. Suddenly, as the unsuspecting coalfish hovered over the head of the angler and sampled [to sample?] the living and actively moving bait—I cannot say that I ever saw it touch the bait with its snout—the angler's mouth would open and as suddenly close upon its prey; the head of the coalfish always disappeared first, while the tail projected from the tightly closed mouth. A few seconds later the tail would be drawn by a sort of suction into the still closed mouth and the angler would be ready for another meal.

The angler was never observed to pursue its prey, but would lie perfectly still on the bottom of the tank. However, when the coalfish were introduced the lure would be "actively jerked to and fro." But when no prey was in sight, the lure would be laid horizontally back on top of the angler's head.

Here then is the long-wished-for proof that the lure is used in fishing. And at long last Aristotle is corroborated.

In 1937, D. P. Wilson published "The Habits of the Angler-fish, *Lophius piscatorius* L., in the Plymouth Aquarium." This account of the habits of a fish, very difficult to maintain in an aquarium, is so full, so clear, so complete, that it is a joy to the student of fish habits and behavior. Of the particular habit of interest here, Wilson gives individual histories of three small specimens which lived in the tanks for months and were often seen to angle with the lure. Another was seen to hold the rod over the closed mouth in readiness to fish but was not actually seen to catch anything.

The whole paper is very interesting, but here is what Wilson writes of the angling:

> An angler when hungry erects the lure immediately any suitable fishes come anywhere near and endeavor to attract one of them close enough to be

caught. The lure is quickly jerked to and fro and, as the rod is almost invisible, the bait (in my specimens always forked and 'fly-like' not vermiform) simulates some tiny creature darting about. An attracted fish rushes up in an endeavor to catch it; the bait is skillfully flicked out of its way just in time and, with a final cast, is dashed down in front of the mouth which may open very slightly. The intended victim, still following the bait, turns slightly head downward; it is now more or less directly head-on to the angler's mouth. The jaws snap faster than the eye can follow and the tail of the prey is next seen disappearing from sight through the firmly closed mouth. As far as I have been able to observe, the bait is not actually touched by the victim before it is caught, as has sometimes been supposed. Touching the bait with forceps does not cause a reflex snapping of the jaws.

The moving lure has a strong attraction for healthy hungry fishes of several kinds. Small pollack, whiting, pout and bass were the species actually observed to be attracted to it, but probably most pelagic fish that capture moving prey by sight would at least swim up to investigate the darting object. Many fish take a ready interest in moving things.

Besides the quick lashing motion of the lure just described, some anglers occasionally combine with it another movement. Every now and then the rod is depressed until the bait hangs just in front of and rather below the level of the lower jaw. It is then for a few minutes given a curious vibratory movement, after which the sharp flicking is again resumed, the bait being jerked over wide arcs in various directions.

The lure, says Wilson, is not always used when prey is to be captured. If a fish happens to swim near the head of the angler it may be engulfed. Anglers vary in the use of the lure—some use it often, some infrequently. When the lure is not in use, it is always laid back between the eyes. "Some individuals were often observed to direct the lure forward horizontally across the mouth, keeping it quite still for hours on end." For this no definite conclusion was arrived at, but hunger was suspected to be the explanation.

The prey, always in front and just above the level of the mouth, is engulfed too rapidly for the human eye to follow. At one moment the prey is trying to seize the bait, the next the angler is sinking down in its sandy bed with the tail of the prey projecting from its mouth, and in the next minute the tail is gone. Thus the fish almost invariably is taken in head first. When the prey is caught broadside on, it has to be worked around until the head goes in first.

These accounts are all very clear and coherent, but some one will make the point that these observations were made of the behavior of small specimens under artificial conditions in aquariums where the possibly semi-starved fish, restricted in its movements, perhaps behaved abnormally. What is wanted, it will be declared, is an observation of the fish using the lure out in free water under natural conditions—as Aristotle's was.

Such an observation was published in 1925, by Bigelow and Welsh in their "Fishes of the Gulf of Maine" under the name *L. piscatorius.* Parenthetically it may be noted that in 1936 Bigelow and Schroeder changed this name to the present designation. The fishing habits of the American angler-fish in open water will now be described under its new-old and correct name:

THE AMERICAN ANGLER, LOPHIUS AMERICANUS, USES ITS LURE IN FISHING

Neither Bigelow nor Welsh was so fortunate as to see this fishing, but they quote an eyewitness.

> W. F. Clapp, who has often watched the feeding habits of goosefish [*Lophius americanus*] in Duxbury Bay, Massachusetts, where they are plentiful, describes them to us as lying perfectly motionless among the eelgrass with the tag or "bait" on the tip of the first dorsal ray swaying to and fro over the mouth, either with the current or some voluntary motion so slight as to be invisible. The only fish he has seen them take are tomcod and when one of these chances to approach it usually swims close up to the "bait" but never (in his observation) actually touches it, for, as soon as the victim is within a few inches, the goosefish simply opens its vast mouth and closes it again, engulfing the victim instantaneously.

Then Bigelow and Welsh make the sound remark that "These observations are the more welcome as no other recent student seems to have seen the feeding habits of this species in its natural surroundings." Indeed, they might properly have omitted "recent." As a matter of fact, so far as my search goes, Clapp seems to be the first man known, since Aristotle, to describe the angler's use of its lure in open ocean water. However, his statement is effectually buried in what purports to be purely a systematic work on the "Fishes of the Gulf of Maine." But as a matter of fact this book is for its individual fishes crammed with good natural history notes.

Furthermore, attention is called to the year of publication of Clapp's data—1925. Had his account of the use of the lure by Lophius in natural surroundings been published separately under its own proper title, it would by four years have antedated Chadwick's published observation of the angling by a Lophius in an aquarium. However, neither Chadwick's title (1929) nor Wilson's (1937) indicates that the lure was used in fishing. *A priori* one would infer that its use was noted in both sets of observations, but one can not know until the articles have been read. The use should have been stated in each title.

It having been proved by eye witnesses that two species of the genus Lophius use the lure in fishing, it may confidently be expected that other species of this genus, which have a well-formed lure, will have the same feeding habits.

The publication of a historical article like this one has several values. It brings together all the data on the particular subject that can be found by careful search. The distinctive title gets the phenomenon definitely cited in the bibliographies. When read, the article establishes the behavior as a natural phenomenon, and may incite others to publish their like observations—to the enrichment of the literature of the habits and behavior of fishes.

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REVIEWS AND COMMENTS

EDITED BY PROFESSOR CARL L. HUBBS

IN these reviews and notices of current biological publications emphasis is given to books and major articles which fall within the special scope of THE AMERICAN NATURALIST, in that they deal with the factors of organic evolution. REVIEWS AND COMMENTS are meant to include also such general discussions, reports, news items and announcements as may be of wide interest to students of evolution. Except as indicated, all items are prepared by Dr. Carl L. Hubbs, Scripps Institution of Oceanography, University of California, La Jolla, California. All opinions are those of the reviewer.

Mainsprings of Civilization. By ELLSWORTH HUNTINGTON. New York: John Wiley and Sons, Inc.; London: Chapman and Hall, Ltd., 1945: i–xii, 1–660, figs. 1–83. $4.75.

ELLSWORTH HUNTINGTON, a leader in the imaginative school of modern geography, has long sought to analyze the interplay of heredity, physical environment and cultural endowment in the determination of human behavior, past and present. In this book he coordinates his previous contributions and adds a wealth of new ideas and facts, in an effort to interpret the fundamental basis of civilization. He draws freely and not inexpertly on the data of evolutionary biology, conventional history, business economics, climatology and other sciences. Although he stretches his vision at times, his views as a rule are judicious and cautious. His more important conclusions are either supported by an extensive mass of critically analyzed data, or are proposed tentatively as working hypotheses. That kind of geography is certainly not to be regarded as unscientific.

The author avoids entanglement in the whirlpool of argument over "races." In the first place he stresses the importance and necessity of environmental and cultural as well as of hereditary factors, without attempting to appraise the relative importance of the three (two?) factors. In the second place he recognizes that racial differences are of much less magnitude than innate individual differences or than differences due to environmental factors, and further emphasizes that each race contains the material out of which progressive advances in civilization are possible under the stimulus of optimum environment and rigorous selection. In the third place he rightly decries the insane concepts of race that were held by the Nazis (and by members of other arrogant groups), and analyzes the fallacies of the sister concepts of geopolitics. In the fourth place he shies away from discussions of races, by substituting "kith" for race. A kith he describes as a human group held together by intermarriage. But in the broader biological sense, which avoids the conventional and the abused concepts of race as applied to man, a kith is a race, with a degree of integrity commensurate with the degree of interbreeding. The fact that a kith may be of diverse origins does not distinguish it from a race, for the race is the unit of speciation and speciation is often reticulate, probably usually so, to some degree. Even so clear a race as the Tasmanian is termed a kith.

A brief review of the evolution of animal life leads to the conclusion that civilization is characterized by the increasing control of the environment and as such represents the continued operation of a basic evolutionary force. The evolution of man through natural selection is related to the circumstance that man in many ways is a very generalized animal. Cultural as well as organic evolution is attributed to selection. Particular stress is laid not only on the rapid racial improvement of stocks (kiths), through selective processes that are inherent in migrations, but also on the century-long perpetuation of such advances. These conclusions are supported by a extended, data-crammed analysis of the superior qualities of the descendents of early pilgrim families (including the Huntingtons), and by a rather convincing though less thoroughly documented discussion of Icelanders, Jews, Junkers, Indian Parsis,. Chinese Hakkas and nomads of various "kiths."

The geographical distribution of civilized qualities in the United States and throughout the world is analyzed and mapped. Consideration is given to the hereditary bases, particularly those that are attributable to selection during migrations. More emphasis, however, is placed on the rôle of climate as a factor in civilization.

Seasonal variations in human efficiency and culture levels are analyzed at length, as further evidence that the environment controls human behavior. Storms are shown to have major effects on physiological and mental functions. Short-term and global, long-term weather cycles are proved to be real and to have had throughout history great effects on health, business activities and cultural levels. The rise and fall of civilizations, as well as lesser and briefer fluctuations in culture, are interpreted as due at least in part to variations in the environment. Temperature and humidity are indicated as of importance but fail to explain many of the features of cyclic behavior that obviously stem from the milieu. The search for other factors has led to the most pregnant suggestions, proposed guardedly though with obvious favor, that atmospheric electricity and ozone are particularly effective in determining man's level of health, activity, success and progress. In true scientific spirit Huntington grasps for more data to test these revolutionary concepts.

The Life History of an American Naturalist. By FRANCIS B. SUMNER, Lancaster, Pa.: The Jaques Cattell Press, 1945: i–viii, 1–298. $3.00.

THE last of the many cherished conversations I had with FRANCIS BERTODY SUMNER so well exemplified his intellectual honesty—a ruling force in his life—that I risk an overly personal touch by recounting the gist of his remarks. During a visit at the local hospital, where he went for observation on one of the several short illnesses that preceded his death on September 6, we naturally discussed his autobiography, which had just come off the press. As I was leaving he called me back from the door to remind me of the frankness that I at times display in reviews and to insist that I allow no personal feelings to inhibit me from expressing exactly what I think of the book. He had told the story of his own life as a vehicle for the expression of his long-considered views on the biology and sociology of man. He had reached out for a wider audience and he yearned for a favorable reception of his ideas, yet he called for unhampered criticism.

In compliance with "F.B."'s last request I will say that some readers, including close friends, take exception to certain views that are expressed in this almost wholly uninhibited and searchingly self-critical autobiography. A few are amazed that he should have analyzed so frankly what he regarded as defects of his own parents, in his effort to estimate the interplay of heredity and environment in determining the weak as well as the strong aspects of his own personality. Others are made unhappy by his repudiation of conventional religious beliefs. Some fellow citizens of southern California will probably dislike the violence of his attack on the booster. Other readers will regret the frankness of his views regarding the unlikable traits which he held to characterize Jews to a greater extent than Gentiles. I imagine there may even be lay readers who will decry his attacks on anti-vivisectionists. A considerable proportion of fellow scientists may ridicule his leanings toward "extrasensory perception" as well as the lingering remnant of his former favorable attitude toward Lamarckism. Despite his keenness and success in experimentation some biologists will belittle his sustained interest in observational natural history.

Such feelings of shock or repugnance I do not share, though I do not wholly agree with all of the views expressed in the thought-crammed book. The unquestionable honesty and frankness of the author smooth down the points of disagreement. When, as often, his ideas lean backward away from conventional viewpoints I appreciate the great force of his free thinking, the rigorousness of his objectivism, and the hypertrophy of his critical judgment, particularly toward himself.

It was obviously these qualities of man and scientist, all revolving about an intense motive of intellectual honesty, combined with extreme modesty, that led Dr. Sumner to portray himself in a light that does not shine with the fullness of truth, in that he obscured his many outstanding virtues. He was a bit reticent but certainly not the retiring and timid soul that is pictured, for he had many warm friends and a host of admirers. To a far greater extent than most scientists do, he courageously engaged in the service of society. He actively furthered the interests of political and social liberalism, calendar reform, animal experimentation, freedom of science, the conduct of pure research, population control, eugenics, nature preservation, free thinking and release from narrow dogma. He was rather deliberate in speech, and in his book makes note of his greater proficiency in writing, but his conversation was eagerly sought and richly enjoyed. Much of his scientific work was, as he stated, detailed, technical and specialized, but it was widely known, often quoted, and very highly regarded for originality and significance. This is notably true of his epochal researches on speciation in mice of the genus *Peromyscus* and of his studies on physiological and evolutionary adaptations in fishes. Election to fellowship in various societies and academies, to the Vice-Presidency of the American Association for the Advancement of Science, and to membership in the American Philosophical Society and the National Academy of Sciences gave evidence of the esteem in which he was held by his colleagues.

This autobiography is delightfully written, with vivid narratives and with visualizing descriptions of personalities and institutions. Choice humor is there, though it may often need seeking by those who were not personally acquainted with the author. The life story, however, merely provides an interest-sustaining background for the dissemination of views on the unwisdom of some generally accepted customs and beliefs—views that are well worth the consideration not only of naturalists, but also of any persons who are intelligently interested in human behavior.

What Is Life? The Physical Aspect of the Living Cell. By ERWIN SCHRÖDINGER. Cambridge: At the University Press; New York: The Macmillan Co., 1945: i–viii, 1–91, figs. 1–12. $1.75.

BEFORE he ventures into the field of biology, in an effort to answer the prime question of life, statistical physicist Schrödinger offers a defense for this act of seeming trespass. He points out the age-old longing for unified, all-embracing knowledge, still felt despite the fact that "it has become next to impossible for a single mind to command more than a small specialized portion of it." He sees "no other escape from this dilemma . . . than that some of us should venture to embark on a synthesis of facts and theories." The apology should be accepted and the author congratulated on his courage as well as insight. The central question he asks is, "How can the events *in space and time* which take place within . . . a living organism be accounted for by physics and chemistry?" "The arrangement of atoms in the most vital parts of an organism and the interplay of these arrangements," he holds to "differ in a fundamental way from all those arrangements of atoms which physicists and chemists have hitherto made the object of their experimental and theoretical research."

The enormous number of atoms in an organism is regarded as consistent with an explanation of its action in terms of statistical physics, though the supposedly somewhat limited number (one or a few million) in a gene renders it difficult, the author thinks, to explain the regularity and permanence of the genetic mechanism. Mutations are interpreted as quantum changes and the physics of life is thereby related to quantum physics. It is held that the permanence of genes leaves no alternative to the theory that genes are molecules. A well-ordered association of atoms is said to be the only conceivable structure that offers a variety of possible arrangements, sufficiently large to embody a complicated system of determinants within a small spatial boundary. Mutations are regarded as isomeric changes in the gene molecules.

In discussing genetics the importance of differences within the normal range of variation is by implication denied, the rôle of gross mutations is overemphasized and DeVriesian mutations, contrary to views now generally held, are presented as typical ones. The necessity of mutation being a rare event is wisely stressed and the relative permanence of the genes is interestingly attributed to natural selection.

Despite the explanations of life functions in terms of statistical and quantum physics, living matter is held probably to involve laws of physics hitherto unknown, which, when revealed, will form an integral part of physical science. The seeming defiance of entropy through metabolism appears to set life apart, and "the unfolding of events in the life cycle of an organism exhibits . . . orderliness, unrivalled by anything we meet in inanimate matter."

The most essential part of the living cell is held to be the chromosome fibre, which is termed *an aperiodic crystal* in contrast with the *periodic crystals* which physicists have hitherto studied. Whether the distinction is as trenchant as the new terminology suggests, the reviewer is not competent to say, though he would point out that chromosomes are unknown in some of the most primitive organisms and that the distinction between living and non-living matter, and hence between biological and physical laws, may be bridged over by considering such lowly forms of life and the viruses.

The Mosquitoes of New Jersey and Their Control. By Thomas J. Headlee. New Brunswick, N. J.: Rutgers University Press, 1945: i–x, 1–326, pls. 1–16, figs. 1–87. $4.00.

Although it is primarily a guide to mosquito control in New Jersey, professional in its main approach and somewhat exuberent in the spirit of a public service well accomplished, this volume will also interest systematic entomologists, students of insect behavior and natural history, ecologists, economic zoologists, wildlife workers and conservation administrators, not to mention thousands of folks whose comfort is shattered and whose health is potentially endangered by the Culicidae.

Correct identification and a knowledge of the life history and flight behavior of each species commonly encountered are stressed as fundamental factors in effective mosquito control. Accordingly, about two hundred pages are devoted to a detailed treatment of the species, under six ecological groups: salt marsh breeding, household, fresh-water swamp, malarial, woodland pool and miscellaneous. The typical treatment gives the scientific and the vernacular name; the range in terms that are too brief and too general; the number collected in New Jersey, from an introductory tabulation of 1,056,628 specimens trapped from 1932 to 1941; hand-lens recognition characters; description of the adult, with a good figure; habits of the adult, with brief accounts of the breeding behavior, biting proclivities, and vagility; description of the larva, particularly detailed and well illustrated; and habits of the early stages, described at length and emphasizing points of concern to ecologists as well as to mosquito control officials. Special emphasis is properly given to the larvae, which are of critical significance in control operations.

This main section is taken bodily from John B. Smith's bulletin of 1904, with slight emendations and small additions. It covers the 37 species of Culicidae other than "*Corethra*" (*Chaoborus*) that were known from New Jersey in 1904, but barely mentions the 4 species that have since been found in the state. It is indeed a tribute to Dr. Smith's energetic and pioneering work that his treatise can be reused, but it would seem that many of the personal observations and reflections could have been abbreviated to make room for further data and conclusions from forty years of mosquito research and control operations.

The first chapter, "Value of Mosquito Control," contains little more than a brief history of the mosquitoes of New Jersey as pests, and leaves to a later chapter the history of control, which is treated from the viewpoint of personalities. Statistical data on the efficacy of mosquito control in New Jersey are so extremely brief as to carry little convincement. Perhaps the results are self-evident to New Jersey residents; but perhaps, as is commonly true in wildlife operations, too small a proportion of the funds and energy expended has gone into a critical evaluation of the results. Almost no consideration is anywhere given to the debit side of the ledger, particularly to the destructive effects that the extensive drainage of marshes may have had on the feeding grounds for waterfowl, on the nursery waters of food and game fishes, or on natural habitats for the pleasurable observation and scientific study of wildlife. In wildlife administration there is obvious need for the joint consideration of all such natural assets.

There can of course be little doubt that the partial elimination of these insect pests has been a great boon in metropolitan and resort areas, but the claims that are made in regard to the financial value of mosquito control in New Jersey appear to credit too much of the rise in property values to such control. It is only natural of course that those who are vigorously and professionally engaged in this socially beneficial activity should see with somewhat biased eyes.

Evidence on the virtual elimination of malaria through mosquito control, in localities where it has reappeared, is presented in the final paragraph of the book, but so briefly as to incite queries in the mind of the critical reader, even though no doubts were left in the author's mind. Otherwise malaria is scarcely mentioned, beyond a five-line passage in the account of the chief vector. Why malaria in New Jersey as in other regions declined to insignificance following a high incidence in early years is not discussed. Nor is there any mention of the possibility that new and virulent types of malaria may be introduced by soldiers returning from malarial areas.

The standard method of mosquito control in the salt marsh areas of New Jersey, where the problem is most acute, is a very interesting example of applied ecology. Briefly the procedure is to alter the marsh drainage, by ditches and tide gates, so as to give the natural enemies of the mosquito larvae access to the mosquito breeding waters at almost all times (but not necessarily for periods shorter than the larval life of the pests). The main enemies are the native killifishes, chiefly species of *Fundulus*. Some efforts have been made to introduce into New Jersey the mosquitofish *Gambusia* which has proved to be a highly effective control agent in many parts of the world. These trial introductions have mostly failed, probably largely because the fish were introduced from too far south. *Gambusia* has recently been successfully acclimated in northern Illinois and in Michigan by a gradual process of the northward extension of its range. The winter-resisting stock thus produced may prove an aid in the further control of mosquitoes in New Jersey and other northern states.

NOTICES OF NEW BOOKS

They Hop and Crawl. By PERCY A. MORRIS. Lancaster, Pa.: The Jaques Cattell Press, 1945: i–xiv, 1–253, many figs. 2nd ed. $3.50.—This most recent of books in the "Humanizing Science" series concerns the descriptions and habits of some of the reptiles and amphibians of the United States—more properly, the eastern United States for the Connecticut-born author draws scarcely at all upon the wealth of material west of the Rockies. The illustrations are excellent, leaving no doubt as to Mr. Morris' superb abilities as a photographer of difficult subjects, and the pronouncedly informal style is readable and non-technical. The great number of errors which marred the first edition (1944) have been substantially reduced; nevertheless there remain a goodly number of minor ones which will bother schooled systematists but will remain undetected, happily, by that great majority for whom the book is obviously intended.—NORMAN HARTWEG, *Museum of Zoology, University of Michigan, Ann Arbor, Mich.*

Optical Instruments. By EARLE B. BROWN. Brooklyn: Chemical Publishing Co., 1945: i–xii, 1–567, figs. 1–211, A–S. $10.00.—The basic principles of design, function and adjustment of the various common types of optical instruments are presented in this volume for the benefit of student, operator, teacher, engineer and repairman, rather than for the optical or mechanical engineer. Considerable space is given to the theoretical discussion of optics, written in a moderately technical but understandable style, with the more complex mathematical proofs relegated to an appendix. There follows a main section on the description, operation and theory of optical instruments—telescope, cameras, microscope, field glasses and binoculars, projectors, spectroscope, theodolites and others, with special treatment of military instruments and range finders. The third part deals with the construction and maintenance of the instruments. Supplementary topics are the design of optical systems, the manufacture of optical glass, of general interest, notes on physical optics. The useful volume ends with a glossary of optical instrument terms and a comprehensive index.

Principles of Modern Biology. By DOUGLAS MARSLAND. A Complete Reconstruction and Modernization of CHARLES R. PLUNKETT's Elements of Modern Biology. New York: Henry Holt and Co., 1945: i–ix, 1–774, figs. 1–350. $3.75.—College teachers who wish to or are willing to displace the natural history or the morphological approach will find in this "reconstructed and modernized" text an excellent aid in elementary instruction in biology. The opening section exposes the student to the principles of nutrition, responsiveness and reproduction and these basic concepts are returned to in subsequent sections. Consistently the emphasis is physicochemical and experimental. Anatomy, to a large extent human, is presented only as scattered adjuncts to the physiological discussions. There is little basis for an appreciation of structural organization, either individual or comparative. Observation is defined as merely the first of four steps in "the scientific method," without any suggestion that some branches of the life sciences—paleontology and human genetics for example—have made great progress in themselves and substantial contributions to general biology, almost entirely without the advantages of controlled experiments.

There is a rather extensive and adequate treatment of heredity, leading to a short consideration of embryonic organizers and the nature of the gene. Speciation is held to be a very gradual process. Natural selection is presented favorably in very general terms, whereas Lamarckism is briefly treated as a disproved theory of evolution. Although labelled "The Consequences of Evolution" the final chapter deals with taxonomy, comparative anatomy, embryology, biochemical relationships and the fossil record as evidences of evolution—all within 27 pages. The classification of plants and animals is relegated to an appendix of 6 pages, followed by a glossary and an index.

A rather high standard of accuracy has been maintained, but a few misleading statements were noted. For example the number of species of chordates is given as 30,000, whereas there are about that many species in the Pisces alone. The increased brain size in vertebrates is illustrated by a figure, to one scale, of the brains of frog, cat and man, without qualification as to size of animal. "Varieties" is used where "subspecies" would be preferable. The electric ray and the "stinging ray" are confused.

As a rule the presentation is superior. The diction is clear and decisive. Choice of subject material has obviously been carefully considered. The explanatory comparisons will catch the interest of modern youth. The text is well illustrated by zinc figures, but the half-tones are dull and lifeless, due perhaps to the war-quality paper. May it soon improve!

SHORTER ARTICLES AND DISCUSSION

SURFACE-MASS RATIO, PALEOCLIMATE AND HEAT STERILITY

For the past few years I have been much interested in temperature trends with respect to the phenomenon of archosaurian extinction, and originally I attributed their disappearance to the possible occurrence of excessively high environmental temperatures acting on the somatic tissue (Cowles, 1939, 1940). In supporting this thesis it was suggested that, because of the climatic conditions presumed to exist as their thermal environment, it was as reasonable to ascribe to fur and feathers the original role of heat exclusion as to insist on their exclusive function as heat-retaining mechanisms. Similarly, the trend in the archosaurian size and changing surface-mass ratio was described as a device to escape deleterious heat effects through the acquisition of progressively smaller surface areas through which to absorb heat and thereby warm the proportionately larger mass of the organisms.

Following the appearance of these articles, objections to the theory were made on the ground that there was no tangible evidence for the existence of either rising or high temperatures and that in fact the consensus of thought agreed on a slowly descending temperature, although the ground for such a supposition seemed open to question.

In spite of the objections based on the absence of tangible evidence for the past existence of high temperatures, the problem continued to receive attention and study, with ultimate result that evidence for thermally induced sterility (Cowles, 1945a, 1945b) accumulated to the point where its apparently universal occurrence could no longer be ignored and this phenomenon of a very heat-sensitive male germ plasm and a more heat-resistant body was substituted for the original concept of heat damage to the body, that is, to the somatic tissues.

Up to the present I have neither received nor seen any objections to this modified viewpoint, but there still remains the question as to whether temperatures had actually risen or fallen during the Cretaceous and immediate post-Cretaceous time.

From the subtle nature of the evidence for this possible climatic trend, it is probable that we must look to biological sources for evidence concerning types of climatic changes that in the nature of things could neither produce nor leave any gross mechanical evidence of their occurrence, such as those left by glaciation.

Up to recently I have been forced to rely in part on the presence in the Cretaceous of corals and tropical vegetation, palm and figs, as far north as Alaska and as far south as Tierra del Fuego to support this theory. Certainly this invasion of areas now characterized by frigid conditions would lead one to suppose that the absence of the tempering effect of a heat-reflecting ice cap, which permitted this invasion, would allow considerably higher temperatures to prevail in what is now called the temperate zone. However, it is commonly contended that the character of plant and animal distribution is merely indicative of the absence of the highly zonal climates of to-day and that the absence of a snow and ice cap with its almost total reflection of heat would not have permitted or resulted in the occurrence of higher temperatures to the south, *i.e.*, would merely have made the climate less zonal.

Even though the distribution of organisms may indicate less zonality than exists at present, there is a reason to suggest that, as would be expected without a frigid polar region, temperatures might also have been high. I refer to the Bergmann principle of size trends in the warm-blooded animals. Of great importance is the complementary but reversed condition that is so apparent in the cold-blooded organism, both vertebrate and invertebrates alike.

Bergmann's principle states in effect that warm-blooded organisms of closely related species increase their size with progressive decreases in the environmental temperatures. This trend is a result of a favorable change in the surface-mass ratio, accompanied by great reduction of the bodies' heat-radiating surface while the heat-producing mass becomes proportionately larger. The saving in energy requirements, *i.e.*, in heat or temperature maintenance, thus tends to foster larger size in colder climates. This effect may be readily envisaged by comparing the rate at which a teaspoonful of hot water will cool to the temperature of the environment, with the rate for a pint, quart or gallon. The underlying principle is the same in both the example and in animals. Therefore, according to the Bergmann principle, because of this differential heat loss, closely related species are larger as they advance into arctic or alpine conditions.

Similarly, while it has been recognized that reptiles decrease in size with progress to colder areas, and that only the smallest lizards or the young of larger but not largest species remain active throughout even southern California's bland desert winters (Cowles and Bogert, 1944; Cowles, 1941), the importance of this trend appears to have been overlooked.

At first glance the logical basis for this trend and the desirability of its incorporation with the Bergmann principle is not apparent, but if one considers the source of heat in the endotherms —the warm-bloods—where excessive heat loss is equivalent to energy loss and exhaustion, with the condition in the ectotherms where heat loss does not represent energy loss, and in which heat is absorbed freely *through the surface,* then in cold climates the favoring effect of small volume and large heat-absorbing surface at once becomes apparent, and one principle complements the other.

Thus it is logical that whenever heat must be absorbed dermally, that is, as in the ectotherms, then the smallest forms of closely related species as in reptilian families, will be found extending their range northward and southward from the equator and its consistently warm climate. That this is the result of favorable changes in the surface-mass ratio can scarcely be doubted.

As has been pointed out in an earlier paper (*supra cit.*) the advantages of small size are numerous and include, amongst other things, a very rapid warming of the body through favorable relationship of large surface in proportion to a relatively small mass and also the ability to use very much smaller food particles which, because of a favorable surface-mass ratio, will digest more rapidly than will the bulkier amounts and sizes of food required for maintenance of larger organisms.

With progression toward the tropics, more abundant and more constant supplies of heat permit an ever-increasing body size in the ectotherms because rapid warming becomes a less important factor owing to the favorable surrounding climate. For these reasons it is only in the tropics, where temperatures are equable and heat-absorption equals heat-loss (because days and nights are equal) that we find our largest reptiles. Almost any family of the Reptilia will suffice for examples, but a few may be mentioned, such as the Iguanidae, Boidae and the Varanidae. These few families are selected because they will probably be familiar to most biologists as well as to workers in other fields.

In the light of facts concerning body size and its relation to the thermal environment, we see that Bergmann's principle with respect to the warm-bloods and also the condition in the reptiles, are both based on the factor of heat economy and heat regulation in terms of favorable changes in the surface-mass ratio.

It is essential to recognize the fact that the trend in the two groups, while apparently opposite in character, is essentially identical because it is based solely on the nature of the heat source, that is, as to whether heat is derived from within through the efforts of the organisms, and must be conserved, or whether the heat is received from without, entailing no energy expenditure. In either case body temperature is obtained or sustained to an effective degree by adjustment through favorable surface-mass ratios.

At this point one may logically ask as to just how these factors fit into an interpretation of paleo-climates. If we apply Bergmann's principle to the warm-bloods in a chronological pattern—linear in time rather than latitudinal in arrangement, we find a steady increase in size in the evolution of such well-known genera as the horses, elephants and other mammals, apparently accompanying falling temperatures that were climaxed by Pleistocene glaciation. Will Bergmann's principle be admitted as pertinent evidence of climatic trends in this case, or will it be rejected? Can one reject the nature of the evidence in the chronological-Paleontological distribution of body size, yet accept it in the case of latitudinal distribution when the parallel is so similar? Then if Bergmann's principle is acceptable with respect to the mammals as applied both to latitudinal and chronological series, what inference can be drawn from the fact of size increase other than that it describes climatic changes? If it is assumed that the similarity is merely accidental and has no bearing on paleo-climatic trends, we must also reject the evidence supporting the Bergmann principle, as well as other no less well-supported ecological principles.

In discussing the Reptilia, the general pattern of a reversed size trend seems to be as reliable, and the principles the same as where the Bergmann principle is applied to the warm-blooded animals. Thus the same surface-mass factor is in operation, and, as we have seen by the trend with respect to falling temperatures, is merely the complementary reverse of that in the warm-bloods, and the explanation is obvious and logical. If then we apply the principle as found in the Reptilia, and use this in attempting to determine temperature trends through the evolutionary development of reptiles, what inferences can be drawn? Can we reject the implication seemingly involved in the size trends in the Reptilia, and consider them inapplicable, while we accept the same factor in the warm-bloods?

It seems reasonable to interpret the enormous size reached by some of the archosaurs as evidence that high temperature must have prevailed in their day just as to-day in living reptiles size increase accompanies temperature increase. Our only other assumption would be that the archosaurs could have conserved even the relatively little internally generated heat and through the exaggerated surface-mass ratios have become essentially "homoiothermous" but not endothermic or warm-blooded. However, it is obvious that this condition could not have eventuated without high environmental temperatures existing while the organisms were en route to this denouement.

The enormous size of a few species must be assumed to have been favored by climatic changes, while other factors such as genetic composition, behavior and change in habits or habitat, that is adjustments as we see them in the living ectotherms to-day, permitted the survival of the smaller species along with the larger forms.

Although the trend towards large size may have reflected climatic conditions, this may not mean that environmental temperatures were exactly proportional to the increase in size, although this may be possible. That is, body temperatures may not have been so extremely high except in the summer when the long days, exceeding by many hours the cooling nights, may have resulted in a daily incremental accession of heat, with a tendency towards ever higher body temperatures. This result might be attained through failure to radiate the daily gain during the short nights.

An important factor that may not be at once apparent is that a size increase of the extent seen in many of the archosaurs may actually represent not a heat-conserving development but a heat-protective device, and that the huge bulk, with resulting small surface area, limited the temperature which they could achieve.

In view of the dependence of the cold-blooded, that is the ectothermic organisms on their environment for a supply of necessary heat and lack of effective protection except through behavior or mass, we can only conclude that environmental temperatures must have been above those of to-day and in summer probably well above the temperature of the tropics. Such conditions must have been necessary in order to permit the development of the massive bodies and relatively small radiation-absorbing areas of archaic reptiles whose size was greater than that of our present-day tropical species. How these animals would have fared in the ancient temperate zone summers is an interesting question.

Whether or not maximum temperatures reached a level at which they would produce deleterious effects in the somatic cells of the reptiles appears to be of relatively little importance because, as noted earlier in this account and as stated more fully elsewhere (*supra cit.*), the notable heat sensitivity of male germ plasm might have been transgressed, and led to sterility or at least to a greatly reduced fertility.

Although we can probably never know whether these ancient forms, like those of to-day, were also characterized by a heat-sensitive germ plasm, its very universality inevitably raises the question as to whether we may have seen in the demise of the archosaurs a possible failure to maintain fertility resulting from either too high an environmental temperature or an incremental temperature rise and prolonged high temperatures in the bodies of these massive ectotherms.

Although many of the conditions needed for final determination of the role of thermal sterility in extinction may never be revealed, no other explanations for size trends and extinction of many forms of life appear to be more clearly in accord with the implications of the few known facts.

In conclusion, I wish to give credit to those colleagues in the Zoology Department who have so considerately discussed the many problems associated with the new views expressed both in this and earlier papers on thermal effects, and who have so generously contributed supporting suggestions and criticism from their special fields. In particular I wish to thank those on whom the heaviest burden of the discussions have fallen, namely, Drs. L. H. Miller, A. W. Bellamy and B. Krichesky.

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THE FORCES INFLUENCING CHROMOSOME PAIRING IN *DROSOPHILA MELANOGASTER*

THE nature of the forces responsible for either the somatic pairing or meiotic synapsis of chromosomes is relatively unknown; no hypothesis accounts adequately for the specificity of the pairing of the homologues. Some investigators assume that a specific attraction exists between all homologous loci which results in the point-to-point pairing. This long-range force is usually thought of as chemical in nature. Fabergé (1942), however, has advanced a hypothesis in which a hydrodynamic phenomenon, the Guyot-Bjerknes effect, is utilized. It is supposed that genes are excited into a state of vibration at the beginning of prophase and have then properties of pulsating spheres; *i.e.,* genes vibrating with the same frequency attract one another. Each gene has its own characteristic frequency so that only its own homologue (or homologues) is attracted. Others think that during random movements of the chromosomes there occur chance meetings of some homologous loci. The synapsis of these loci is then followed by a zipper-like closing up of the rest of the chromosome. Lindegren and Bridges (1938) compare the synapsis of chromomeres to the agglutination of bacteria after the addition of antibodies; when molecules of the specific antibody are absorbed by the corresponding chromomeric surface, such chromomeres become capable of adhering to their homologue or homologues. Loci next to agglutinated chromomeres are then likely to touch, and synapsis of the entire chromosome follows.

Evidence concerning the forces involved in synapsis may be obtained by studies on the pairing of larval salivary gland chromosomes of *Drosophila melanogaster* heterozygous for translocations of different lengths. If synapsis is due to specific attraction between homologous loci, one would not expect translocations of different lengths to affect the normal pairing of the chromosomes, provided the homologous segments of the chromosomes were not too far removed in the nucleus to have an effect on each other; the specific attraction between two chromomeres would be expected to exist regardless of the position of the chromomeres in the chromosome complement. Translocations of different length, then, would be expected to show equal frequency of pairing. If the fusion of chromomeres is the result of chance meetings through random movements, however, one would expect a short translocation to be less frequently synapsed than a longer one, for the larger the piece, the greater the chance of at least one pair of chromomeres coming into contact.

Pairing was observed in two stocks heterozygous for a short translocation and in one stock heterozygous for a long translocation in the X-chromosome. In all three cases, the translocation was between the tip of the X and one arm of the second chromosome, the break in the latter being in or next to the chromocenter so that a very short chromosome, the tip of the X, and a very long chromosome, the rest of the X plus the one arm of the second chromosome, were formed.

In both stocks with the short translocation the break in the X-chromosome is at the white locus. In stock white$^{258\text{-}34}$ the translocation is between the tip of the X and 2R. The salivary gland chromosomes show the break in the X between 3C3 and 3C5, and in 2R in the chromocenter at 41A. In the second stock, w$^{258\text{-}39}$, the tip of the X is translocated to 2L, the break in the X being between 3C3 and 3C5 as in w$^{258\text{-}34}$, while the break in 2L is in the chromocenter at 40E and F.

A stock with the break in the X at the cut locus between 7B2 and 7B5 was used as material for the long translocation. The break in the reciprocal chromosome, 2R, is next to the chromocenter between 41E2 and 41E4–5. This stock is designated as ct$^{268\text{-}17}$. All three stocks were obtained by Demerec from x-rayed males (Bridges and Brehme, 1944).

To minimize variable factors, larvae of these three stocks were used which had the normal homologues of the translocated chromosomes from the wild-type Swedish b–6 stock which had been used as the control for the counts. Preparations were stained with aceto-orcein. Determinations of unsynapsed regions were made according to Bridges' 1935 map.

Since 100 nuclei were observed for each stock, figures in the summarized table represent percentages of synapsed and unsynapsed chromosomes as well as actual numbers. For the X tip, translocations w^{258-34} and w^{258-39} gave approximately the same results, each stock showing only 4 per cent. complete synapsis.

TABLE OF ASYNAPSIS

<table>
<tr><th>Chromosome</th><th colspan="3">Wild-type</th><th colspan="3">w²⁵⁸⁻³⁴</th><th colspan="3">w²⁵⁸⁻³⁹</th><th colspan="3">ct²⁶⁸⁻¹⁷</th></tr>
<tr><td>X Tip</td><td>Complete Synapsis</td><td>Partial Synapsis</td><td>Complete Asynapsis</td><td>Complete Synapsis</td><td>Partial Synapsis</td><td>Complete Asynapsis</td><td>Complete Synapsis</td><td>Partial Synapsis</td><td>Complete Asynapsis</td><td>Complete Synapsis</td><td>Partial Synapsis</td><td>Complete Asynapsis</td></tr>
<tr><td>Sect. 1—3C3-5</td><td>99</td><td>1</td><td>0</td><td>4</td><td>4</td><td>92</td><td>4</td><td>0</td><td>96</td><td>..</td><td>..</td><td>..</td></tr>
<tr><td>Sect. 1—7B2-5</td><td>97</td><td>3</td><td>0</td><td>..</td><td>..</td><td>..</td><td>..</td><td>..</td><td>..</td><td>14</td><td>28</td><td>58</td></tr>
<tr><td>X Body</td><td colspan="2">Asynapsis</td><td>Synapsis</td><td colspan="2">Asynapsis</td><td>Synapsis</td><td colspan="2">Asynapsis</td><td>Synapsis</td><td colspan="2">Asynapsis</td><td>Synapsis</td></tr>
<tr><td>Sect. 3C3-5—20</td><td colspan="2">91</td><td>9</td><td colspan="2">94</td><td>6</td><td colspan="2">91</td><td>9</td><td colspan="2">..</td><td>..</td></tr>
<tr><td>Sect. 7B2-5—20</td><td colspan="2">91</td><td>9</td><td colspan="2">..</td><td>..</td><td colspan="2">..</td><td>..</td><td colspan="2">87</td><td>13</td></tr>
<tr><td>2L</td><td colspan="2">97</td><td>3</td><td colspan="2">88</td><td>12</td><td colspan="2">15</td><td>85</td><td colspan="2">83</td><td>7</td></tr>
<tr><td>2R</td><td colspan="2">89</td><td>11</td><td colspan="2">20</td><td>80</td><td colspan="2">89</td><td>11</td><td colspan="2">17</td><td>83</td></tr>
<tr><td>3L</td><td colspan="2">87</td><td>13</td><td colspan="2">87</td><td>13</td><td colspan="2">88</td><td>12</td><td colspan="2">85</td><td>15</td></tr>
<tr><td>3R</td><td colspan="2">89</td><td>11</td><td colspan="2">92</td><td>8</td><td colspan="2">91</td><td>9</td><td colspan="2">88</td><td>12</td></tr>
</table>

In ct^{268-17}, there was 14 per cent. complete synapsis. To test the significance of this difference the formula $t = \frac{p_1 - p_2}{\sqrt{\varepsilon_1^2 + \varepsilon_2^2}}$ (Yule and Kendall, 1937, p. 360) was applied.

The difference was found to be probably not significant. When the data are broken down into cases of partially unsynapsed and completely unsynapsed tips, however, the difference is obviously significant, there being 28 cases of partial synapsis in the ct stock and only 4 in w^{258-34} and 0 in w^{258-39}, and only 56 cases of completely unsynapsed tips in ct^{268-17} as compared with 92 and 96, respectively, in w^{258-34} and w^{258-39}. The pairing of the reciprocal chromosomes of the translocation was equally affected in each stock, 2R giving only 20 per cent. synapsis in w^{258-34}, 17 per cent. in ct^{268-17} and 2L giving 15 per cent. in w^{258-39}. Pairing in the X body and in the chromosomes not involved in the translocation was not affected in any of the translocated stocks.

Though the increase in the occurrence of complete synapsis in the X tip of the long translocation as compared with that in the short translocation was not found to be significant, the much greater frequency of partial synapsis found in the X tip of the long translocation is definitely significant. The results are an indication that the pairing of chromosomes is the result of the chance meetings of some homologous loci during random movement of the chromosomes, rather than the result of a long-range attraction between chromomeres.

The writer is indebted to Dr. K. S. Brehme for her suggestion of the problem and her guidance throughout the course of its study.

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SEASONAL CYCLES AND THE CONCENTRATION OF INVERSIONS IN POPULATIONS OF *DROSOPHILA FUNEBRIS*

THE question whether the living conditions to which a species is subject may determine the degree of saturation of a population by chromosomal variants was first raised by Dubinin, Socolov and Tiniakov (1937). Dobzhansky (1943) first showed the direct influence of selection upon inversions in natural populations of *Drosophila pseudoobscura* and demonstrated the dependence of varying concentrations on seasonal cycles.

From the material of our work of 1937, it follows that in the winter (December) population of *Drosophila funebris* in 1936 the concentration of inversion heterozygotes was 75.66 per cent., while in August, the period when the same populations had reached its maximum, the concentration was 107.98 per cent.

In 1944 and 1945 three populations within the city of Moscow were examined monthly for the proportions of the inversion II–1. The results appear in Table 1.

These data demonstrate clearly that changes occur in the concentration of inversions in accord with the seasonal cycle. As the populations increased from small densities of spring to enormous sizes at the end of the summer and the beginning of autumn, there occurred a perceptible increase of inversion concentrations. For the Hippodrome population the increase from May to August was 15 times, for the population of the Botanical Garden from May to August 1.8 times, for the Voronzovo Polye from March to September 3.7 times.

TABLE 1

PERCENTAGE OF INVERSIONS FOUND

	Localities*			
Month	A	B	C	D
March	5.05	....	13.48	...
April	2.72	....	27.73	...
May	0.98	33.72	....	3.44 (n = 29)
June	6.25	44.00	....	0.42 (n = 234)
July	9.42	52.00	43.11	
August	14.75	57.58	41.86	1.70 (n = 530)
September	....	53.19	50.84	0.76 (n = 255)
October	....	....	....	1.68 (n = 238)
November	8.53	....	....	...
Individuals	1,045	501	869	1,286

* A—Hippodrome; B—Botanical Garden of the Moscow State University; C—Obukh Street (Voronzovo Polye); D—the village of Kropotovo.

We have also shown that sharp differences exist in the degree of saturation by inversions of urban and rural populations of *Drosophila funebris* (Dubinin and Tiniakov, 1946). It is of interest that we have not observed seasonal changes of inversion concentrations in the rural populations. At Kropotovo (115 km. southeast of Moscow) we have the following proportions of heterozygotes for three inversions (II–1, II–2, IV–1) in different months of the population cycle (see locality D in Table 1). A total of 1,286 individuals was studied; the average proportion of heterozygotes was 1.26 per cent. The different genotypical reactions which urban and rural populations of *D. funebris* exhibit during the progress of the seasonal cycle is of interest, since they indicate hereditary divergence in the process of evolution of urban and rural races of this species.

The facts set forth above give evidence of an energetic selection which has changed the genetic structure of populations in response to changes in habitats. Contrary to the prevailing view that evolution proceeds very slowly, we have observed rapid changes.

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